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(54) Title: SELECTIVE CYCLIC PEPTIDES

(57) Abstract: The present invention refers to peptides cyclized via disulfide or lactam bridges having melanocortin-4 receptor (MC4-R) agonist activity useful for treatment of obesity.

Selective Cyclic Peptides

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Obesity is widely recognized as a serious health problem for the developed countries, and has reached epidemic status in the United States. More than 50% of the U.S. population is considered overweight, with >25% diagnosed as clinically obese and at considerable risk for heart disease, non-insulin dependent diabetes mellitus (NIDDM), hypertension, and certain cancers.

- 10 This epidemic presents a significant burden on the health care system as projected obesity treatment costs of more than \$70 billion annually are expected in the U.S. alone. Strategies for treating obesity include reducing food intake or enhancing the expenditure of energy.

It has been demonstrated that, when injected into the third ventricle of the brain or intraperitoneally, a cyclic heptapeptide analog of α -melanocyte stimulating hormone (α MSH) having melanocortin-4 receptor (MC4-R) agonist activity caused long lasting inhibition of food intake in mice. This effect was reversible when co-administered with a MC4-R antagonist. (Fan, et al., Nature (1997) 385: 165-168) Therefore, agonists of MC4-R activity would be useful in treating or preventing obesity.

There are five known melanocortin receptors based on sequence homology that ranges from 35-60% homology between family members ((Cone, et al., Rec. Prog. Hormone Res. (1996) 51: 287-318), but these receptors differ in their functions. For example, the MC1-R is a G-protein coupled receptor that regulates pigmentation in response to the α MSH, which is a potent agonist of MC1-R. (Cone, et al., *ibid.*). Agonism of the MC1-R receptor results in stimulation of the melanocytes which causes eumelanin and increases the risk for cancer of the skin. Agonism of MC1-R can also have neurological effects. Stimulation of MC2-R activity can result in carcinoma of adrenal tissue. The effects of agonism of the MC3-R and MC5-R are not yet known. All of the melanocortin receptors respond to the peptide hormone class of melanocyte

- 2 -

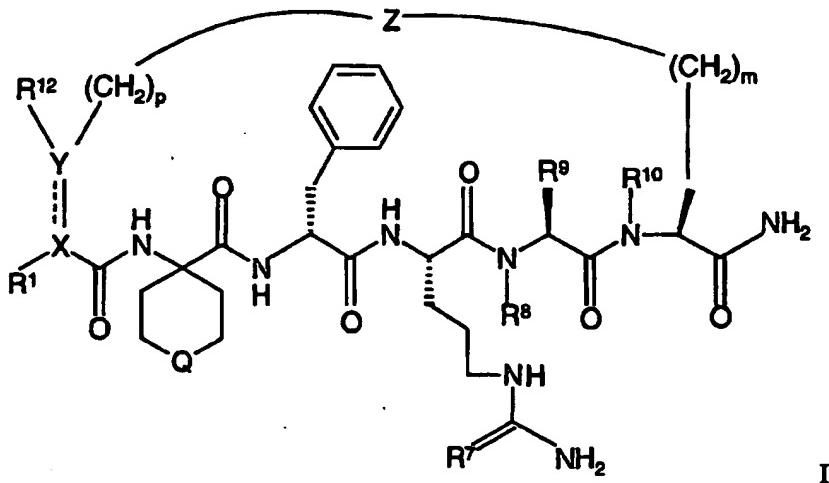
stimulating hormones (MSH). These peptides are derived from pro-opiomelanocortin (POMC), a prohormone of 131 amino acids that is processed into three classes of hormones; the melanocortins (α , β and γ), adrenocorticotropin hormone (ACTH), and various endorphins (e.g. lipotropin) (Cone, et al., *ibid.*). Because of their different functions, simultaneous agonism of the activities of multiple melanocortin receptors has the potential of causing unwanted side effects. Therefore it is desirable that an agonist of MC4-R be more selective for the MC4-R than for one or more of the other melanocortin receptors.

Haskell-Luevano, et al. (Peptides (1996) 17(6): 995-1002) disclose peptides that contain the tripeptide (D)Phe-Arg-Trp and exhibit melanotropic (skin darkening) activity in the frog 10 (*Rana pipiens*) skin bioassay. Haskell-Luevano, et al. (*ibid.*) do not disclose any compound of formula I or II described below.

Bednarek, et al. (Peptides (1999) 20: 401-409) and Bednarek, et al. (Biochem. Biophys. Res. Comm. (1999) 261: 209-213) disclose analogs of the cyclic peptide MT-II. They do not disclose any compound of formula I or II described below.

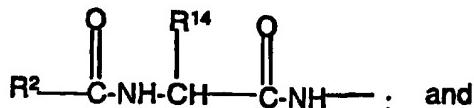
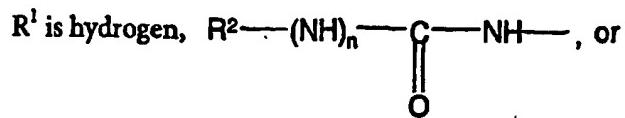
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This invention provides a compound of the formula:

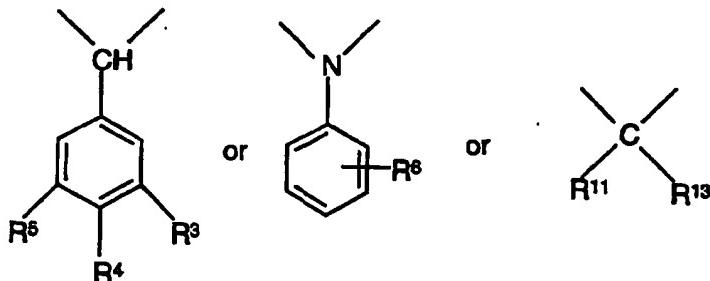


20 In compounds of formula I, R¹ and R¹² together with X and Y form a phenyl ring and X is C and Y is C; or

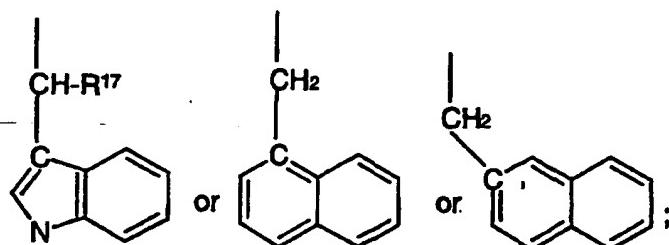
- 3 -



- R^{12} is hydrogen; with either X and Y being each C and the bond between X and Y being a double bond, or with X and Y being each CH and the bond between X and Y being a single bond; R^2 is alkyl having from 1 to 5 carbon atoms, alkenyl having from 2 to 5 carbon atoms, or alkynyl having from 2 to 5 carbon atoms; R^{14} is alkyl having from 1 to 5 carbon atoms; and n is 0 or 1, Q is
- 5

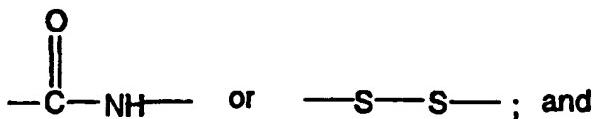


- 10 wherein R^3 , R^4 and R^5 are independently hydrogen, halo, alkyl having from 1 to 4 carbon atoms, hydroxy or alkoxy having from 1 to 4 carbon atoms, wherein when R^4 is not hydrogen, R^3 and R^5 are both hydrogen; R^6 is hydrogen, alkyl having from 1 to 3 carbon atoms, alkoxy having from 1 to 3 carbon atoms, phenoxy, or halo; and R^{11} and R^{13} are each independently hydrogen, alkyl having 3 or 4 carbons, or cycloalkyl having 5 or 6 carbon atoms or R^{11} and R^{13} are both phenyl;
- 15 R^7 is O or NH; R^8 is hydrogen or methyl. R^9 is



R^{10} is hydrogen or methyl; p is 0 or 1; m is 0, 1, 2, or 3; Z is

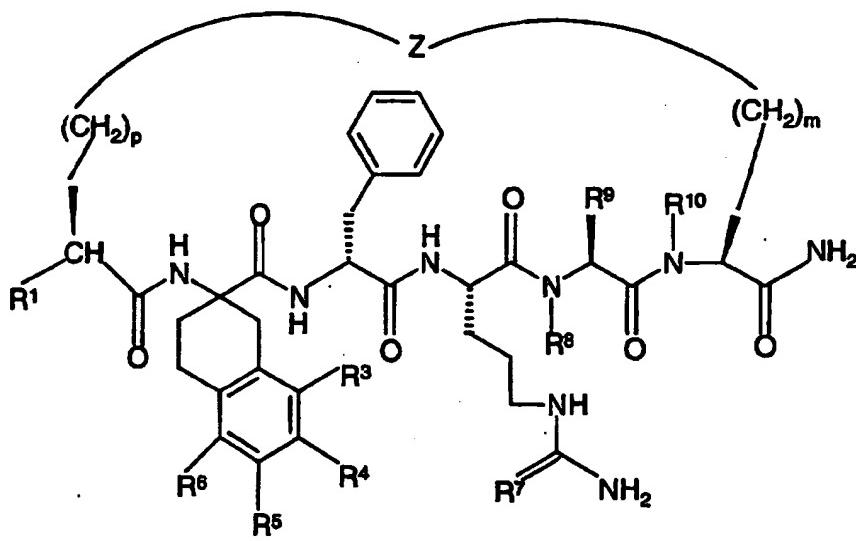
- 4 -



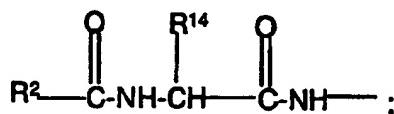
R^{17} is hydrogen or lower alkyl, preferably methyl and pharmaceutically acceptable salts thereof.

- The dotted bond in the compound of formula I is hydrogenated when X and Y are each -CH-. On the other hand, when the dotted bond is present, Y and X taken together with R^1 and R^{12} do not form a phenyl ring, and X and Y are both tetravalent C atoms.

This invention also provides a compound of the formula:

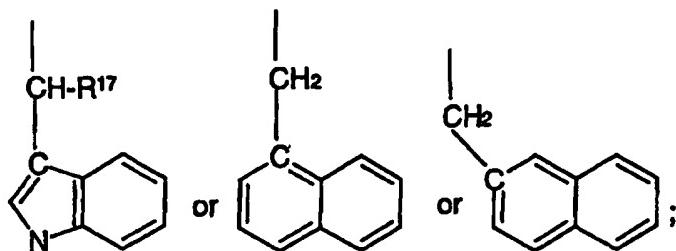


In compounds of formula II, R^1 is hydrogen, R^2 — $(\text{NH})_n$ — $\text{C}(=\text{O})$ — NH —, or

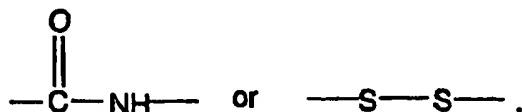


- 10 R^2 is alkyl having from 1 to 5 carbon atoms, alkenyl having from 2 to 5 carbon atoms, or alkynyl having from 2 to 5 carbon atoms; R^{14} is alkyl having from 1 to 5 carbon atoms; and n is 0 or 1, one of R^3 , R^4 , R^5 and R^6 is hydrogen, halo, alkyl having from 1 to 3 carbon atoms, or alkoxy having from 1 to 3 carbon atoms, and the remainder are hydrogen. R^7 is O or NH. R^8 is hydrogen or methyl. R^9 is

- 5 -



R^{10} is hydrogen or methyl; p is 0 or 1; m is 0, 1, 2, or 3; Z is



R^{17} is hydrogen or lower alkyl, preferably methyl and pharmaceutically acceptable salts thereof.

- 5 The compounds of formulas I and II as well as Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Ala-Trp-Lys-NH₂; and Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Arg-(2S,3S) beta methyl-Trp-Lys-NH₂ are agonists of the MC4-R. It is known that agonists of MC4-R activity cause reduction of food intake in a mouse model of human obesity. Therefore these compounds are useful in the treatment or prevention of obesity.
- 10 All of the compounds of formulas I and II exemplified below as well as Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Ala-Trp-Lys-NH₂; and Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Arg-(2S,3S) beta methyl-Trp-Lys-NH₂ were tested for MC4-R agonist activity and MC1-R agonist activity in the *in vitro* assay described below in Biological Activity Example A. All of the tested compounds had an EC50 for MC4-R agonist activity of less than 500 nM, and all exhibited at least 10-fold greater MC4-R agonist activity than MC1-R agonist activity. In contrast, the compound Ac-Nle-Cyclo(Asp-Lys)-Asp-His-(D)Phe-Arg-Trp-Lys-NH₂ exhibited about equal MC1-R and MC4-R agonist activities.
- 15

Nomenclature and Abbreviations

- 20 The term "alkyl" means a straight chain or branched alkyl group and the term "lower alkyl" denotes an alkyl group containing from 1 to 6 carbon atoms. The term "alkenyl" means a straight chain or branched alkenyl group. The term "alkynyl" refers to a straight chain or branched alkynyl group.

- 6 -

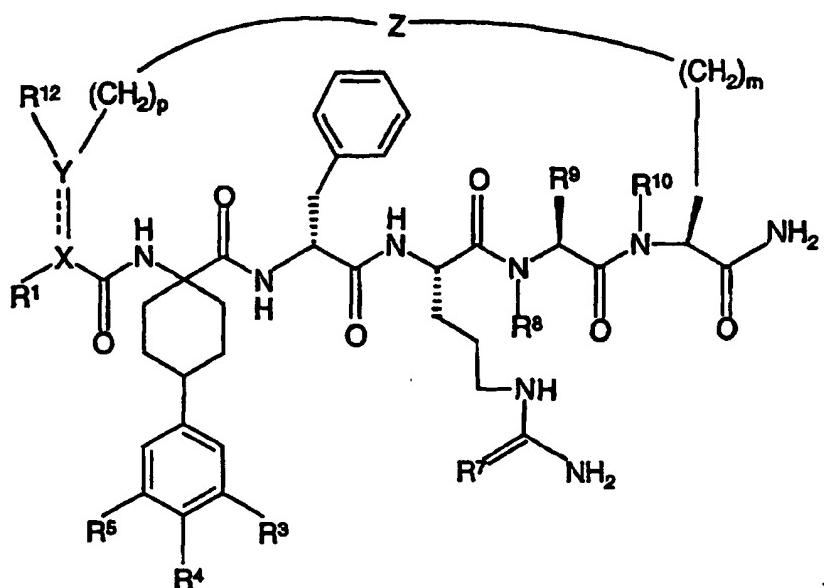
The term "alkoxy" means a group of the formula alkyl-O- in which alkyl is as defined above. The term "phenoxy" means a group of the formula phenyl-O-. Except where otherwise specified, "phenyl" refers to an unsubstituted phenyl ring and "phenoxy" refers to an unsubstituted phenoxy group.

- 5 The term "halo" means a group selected from fluoro, chloro, bromo, and iodo.

The term "pharmaceutically acceptable salt" refers to those salts which retain the biological effectiveness and properties of the free bases or free acids, which are not biologically or otherwise undesirable. The salts are formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, 10 propionic acid, glycolic acid, pyruvic acid, oxylic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, N-acetylcysteine and the like. In addition these salts may be prepared form addition of an inorganic base or an 15 organic base to the free acid. Salts derived from an inorganic base include, but are not limited to, the sodium, potassium, lithium, ammonium, calcium, magnesium salts and the like. Salts derived from organic bases include, but are not limited to salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, lysine, arginine, N-ethylpiperidine, piperidine, 20 polymine resins and the like.

Compounds of formula IA are represented as follows:

-7-

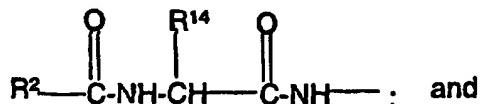


1A

wherein R¹, R³, R⁴, R⁵, R⁷, R⁸, R⁹, R¹⁰, R¹², X Y, Z, m and p are as above and pharmaceutically acceptable salts thereof.

In the compounds of formula IA, R¹ and R¹² together with X and Y form a phenyl ring; or

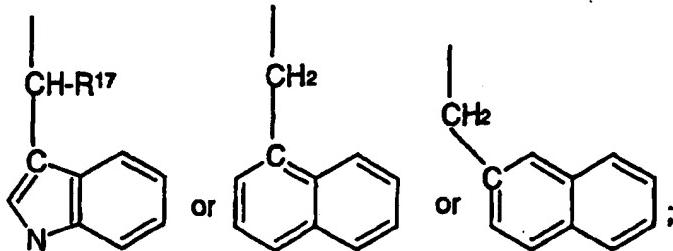
- 5 R¹ is hydrogen, R²—(NH)_n—C—NH—, or



R^{12} is hydrogen, with either X and Y being each C and the bond between X and Y being a double bond, or with X and Y being each CH and the bond between X and Y being a single bond; R^2 is alkyl having from 1 to 5 carbon atoms, alkenyl having from 2 to 5 carbon atoms, or alkynyl

- 10 having from 2 to 5 carbon atoms; R^{14} is alkyl having from 1 to 5 carbon atoms; and n is 0 or 1. R^3 , R^4 and R^5 are independently hydrogen, halo, alkyl having from 1 to 4 carbon atoms, hydroxy, or alkoxy having from 1 to 4 carbon atoms; wherein when R^4 is not hydrogen, R^3 and R^5 are both hydrogen. R^7 is O or NH. R^8 is hydrogen or methyl. R^9 is

- 8 -



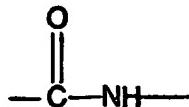
R^{10} is hydrogen or methyl; p is 0 or 1; m is 0, 1, 2, or 3, Z is



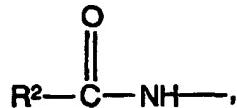
R^{17} is hydrogen or lower alkyl, preferably methyl.

5 The dotted bond in formula IA can be hydrogenated. When the dotted line is hydrogenated X and Y are both $-CH-$. On the other hand, when the dotted bond is present, Y and X taken together with R^1 and R^{12} do not form a phenyl ring, X and Y are both the tetravalent C atoms.

In one embodiment of the compound of formula IA, X and Y are each CH and the bond
10 between X and Y is hydrogenated to a single bond; Z is

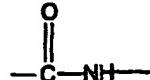


R^7 is O; R^1 is



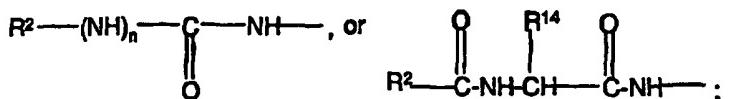
R^2 is alkyl having from 1 to 5 carbon atoms; and R^{10} and R^{12} are both hydrogen. Examples of
15 such compounds include Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Cit-Trp-Lys-NH₂.

In another preferred embodiment of the compound of formula IA Z is



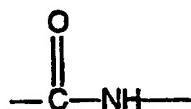
R^7 is NH; R^1 is hydrogen,

- 9 -

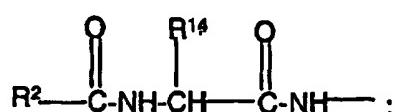


R^2 is alkyl; and R^{10} and R^{12} are both hydrogen.

In another embodiment of the compound of formula IA Z is

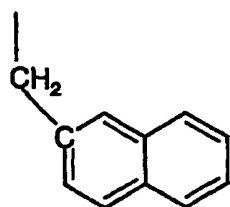


R^7 is NH; R^1 is



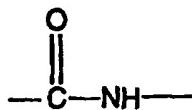
R^2 is alkyl; and R^{10} and R^{12} are both hydrogen.

In a more specific embodiment, X and Y are each CH and the bond between X and Y is
10 hydrogenated to a single bond; n is 0; and R^9 is



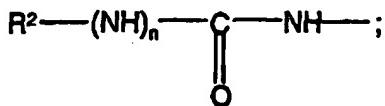
Examples of such compounds include: Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Arg-(2)Nal-Lys-NH₂; and penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Arg-N-methyl(2)Nal-Lys-NH₂.

In another more specific embodiment of the compound of formula IA, Z is

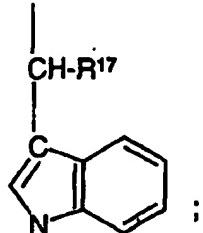


R^7 is NH; R^1 is

- 10 -



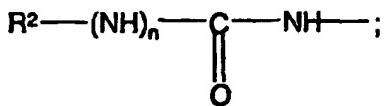
R^2 is alkyl; and R^{10} and R^{12} are both hydrogen; and R^9 is



5 and R^{17} is hydrogen or lower alyl, preferably methyl.

Such compounds include those in which X and Y are each CH and the bond between X and Y is a single bond; and one of R^3 , R^4 and R^5 is hydrogen, halo or alkyl and the remainder are hydrogen, for example Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Arg-Trp-Lys-NH₂; Penta-cyclo(Asp-Lys)-Asp-4-MeApc-(D)Phe-Arg-Trp-Lys-NH₂; Penta-cyclo(Glu-Lys)-Glu-Apc-(D)Phe-Arg-Trp-Lys-NH₂; Penta-cyclo(Asp-Orn)-Asp-Apc-(D)Phe-Arg-Trp-Orn-NH₂; Penta-cyclo(Asp-Dbr)-Asp-Apc-(D)Phe-Arg-Trp-Dbr-NH₂; Penta-cyclo(Asp-Dpr)-Asp-Apc-(D)Phe-Arg-Trp-Dpr-NH₂; and Ac-cyclo(Asp-Dpr)-Asp-Apc-(D)Phe-Arg-Trp-Dpr-NH₂.

Another specific embodiment of the compounds of formula IA, are those compounds in which X and Y are each CH and the bond between X and Y is a single bond; and R^1 is

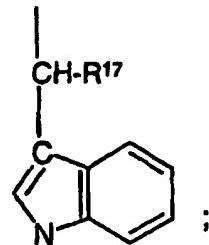


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one of R^3 , R^4 and R^5 is alkoxy, and the remainder are hydrogen; and n is 0, for example Penta-cyclo(Asp-Lys)-Asp-4-MeOAp-(D)Phe-Arg-Trp-Lys-NH₂; Penta-cyclo(Asp-Lys)-Asp-4-EtOAp-(D)Phe-Arg-Trp-Lys-NH₂; Penta-cyclo(Asp-Lys)-Asp-4-iPrOAp-(D)Phe-Arg-Trp-Lys-NH₂; Penta-cyclo(Asp-Lys)-Asp-3-MeOAp-(D)Phe-Arg-Trp-Lys-NH₂; Penta-cyclo(Asp-Lys)-Asp-4-OHAp-(D)Phe-Arg-Trp-Lys-NH₂; and Penta-cyclo(Asp-Lys)-Asp-4-ClAp-(D)Phe-Arg-Trp-Lys-NH₂.

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Embodiments of compounds of formula IA include those compounds in which each of R¹, R³, R⁴, R⁵, R⁸ and R¹⁰ is hydrogen; R⁷ is NH; R⁹ is

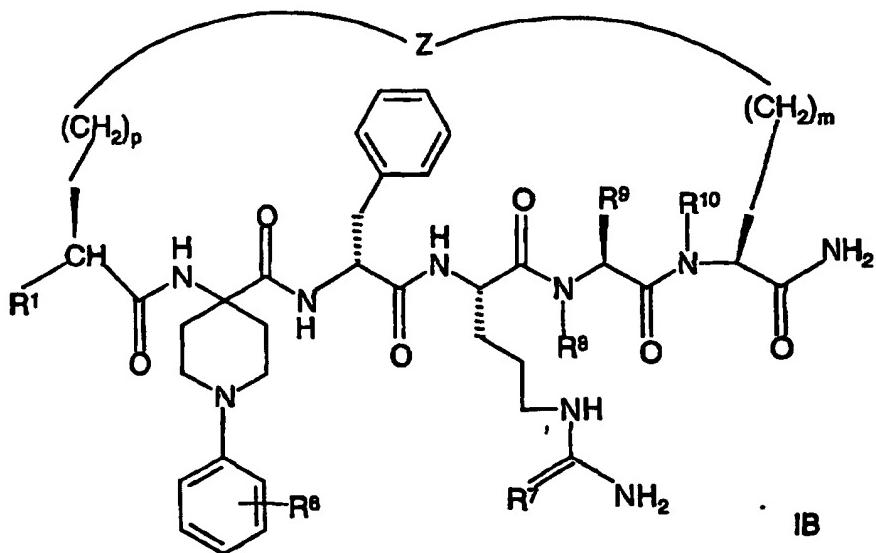


and R¹⁷ is hydrogen or lower alkyl preferably methyl;

- 5 and p is 0, for example cyclo(succinic acid-Lys)-succinic acid-Apc-(D)Phe-Arg-Trp-Lys-NH₂, cyclo(maleic acid-Lys)-maleic acid-Apc-(D)Phe-Arg-Trp-Lys-NH₂, cyclo(succinic acid-Dpr)-succinic acid-Apc-(D)Phe-Arg-Trp-Dpr-NH₂, cyclo(maleic acid-Dpr)-maleic acid-Apc-(D)Phe-Arg-Trp-Dpr-NH₂.

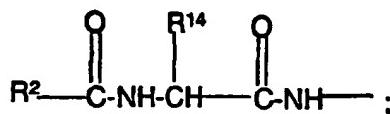
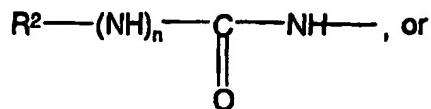
In another embodiment of the compound of formula IA, R¹ and R¹² together with X and Y
10 form a phenyl ring. Examples of such compounds include Cyclo(phthalic acid-Lys)-phthalic acid-Apc-(D)Phe-Arg-Trp-Lys-NH₂; Cyclo(phthalic acid-Dpr)-phthalic acid-Apc-(D)Phe-Arg-Trp-Dpr-NH₂; and Ac-Nle-cyclo(Cys-Cys)-Cys-Apc-(D)Phe-Arg-Trp-Cys-NH₂.

Compounds of formula IB are represented by the formula:



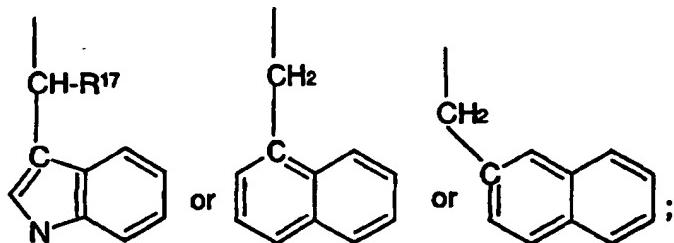
- 12 -

In the compounds of formula IB, R¹ is hydrogen,



5

R² is alkyl having from 1 to 5 carbon atoms, alkenyl having from 2 to 5 carbon atoms, or alkynyl having from 2 to 5 carbon atoms. R¹⁴ is alkyl having from 1 to 5 carbon atoms. N is 0 or 1. R⁶ is hydrogen, alkyl having from 1 to 3 carbons, alkoxy having from 1 to 3 carbons, phenoxy, or halo; R⁷ is O or NH. R⁸ is hydrogen or methyl. R⁹ is



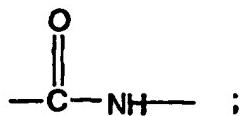
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R¹⁰ is hydrogen or methyl; p is 0 or 1; m is 0, 1, 2, or 3; Z is



R¹⁷ is hydrogen or lower alkyl, preferably methyl and pharmaceutically acceptable salts thereof.

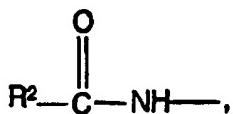
In an embodiment of the compounds of formula IB, i.e., compounds of formula IB1, Z is



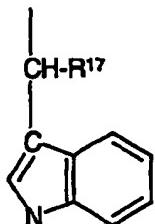
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R⁷ is NH; R¹ is

- 13 -



R^2 is alkyl; R^8 and R^{10} are each hydrogen; and R^9 is



5 and R^{17} is as above

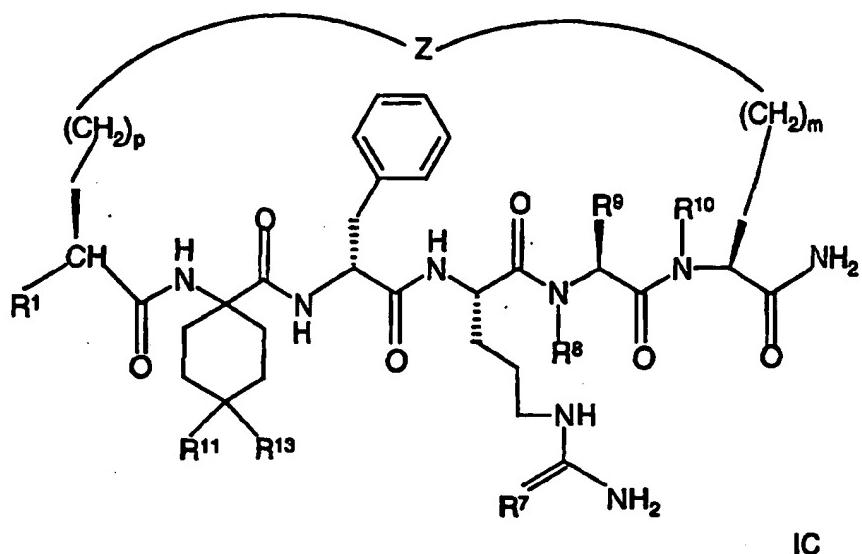
In a more specific embodiment of such compounds of formula IB1, R^6 is hydrogen or alkyl. Examples of such compounds include: Penta-cyclo(Asp-Lys)-Asp-Appc-(D)Phe-Arg-Trp-Lys-NH₂; Penta-cyclo(Asp-Lys)-Asp-2-MeAppc-(D)Phe-Arg-Trp-Lys-NH₂; Penta-cyclo(Asp-Lys)-Asp-2-iPrAppc-(D)Phe-Arg-Trp-Lys-NH₂; Penta-cyclo(Asp-Lys)-Asp-3-MeAppc-(D)Phe-10 Arg-Trp-Lys-NH₂; and Penta-cyclo(Asp-Lys)-Asp-4-MeAppc-(D)Phe-Arg-Trp-Lys-NH₂.

In another more specific embodiment of such compounds of formula IB1, R^6 is halo. Examples of such compounds include Penta-cyclo(Asp-Lys)-Asp-4-ClAppc-(D)Phe-Arg-Trp-Lys-NH₂.

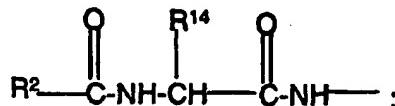
In another more specific embodiment of such compounds of formula IB1, R^6 is alkoxy or phenoxy. Examples of such compounds include: Penta-cyclo(Asp-Lys)-Asp-4-PhOAppc-(D)Phe-Arg-Trp-Lys-NH₂; and Penta- (Asp-Lys)-Asp-3-MeO-Appc-(D)Phe-Arg-Trp-Lys- NH₂.

Compounds of formula IC are represented as follows:

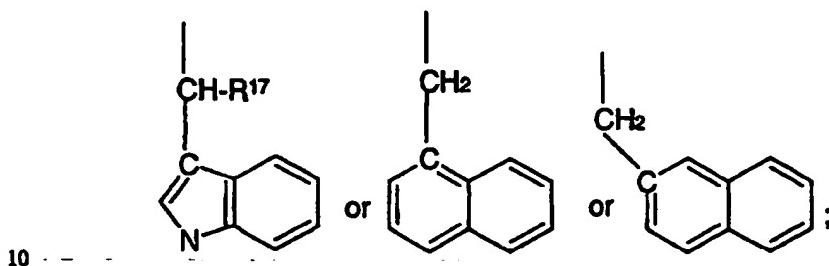
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In the compounds of formula IC, R¹ is hydrogen or



- 5 R² is alkyl having from 1 to 5 carbon atoms, alkenyl having from 2 to 5 carbon atoms, or alkynyl having from 2 to 5 carbon atoms. R¹⁴ is alkyl having from 1 to 5 carbon atoms. n is 0 or 1, R¹¹ and R¹³ are each independently hydrogen, alkyl having 3 or 4 carbon atoms, cycloalkyl having 5 or 6 carbon atoms or R¹¹ and R¹³ are both phenyl; R⁷ is O or NH; R⁸ is hydrogen or methyl; R⁹ is



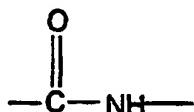
R¹⁰ is hydrogen or methyl; p is 0 or 1; m is 0, 1, 2, or 3; and Z is



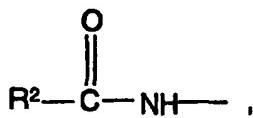
- 15 -

R^{17} is hydrogen or lower alkyl, preferably methyl and pharmaceutically acceptable salts thereof.

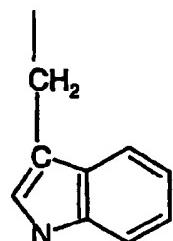
In an embodiment of the compound of formula IC, the compound of formula IC1, Z is



R^7 is NH; R^1 is



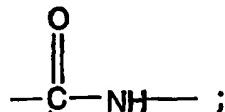
R^2 is alkyl; R^8 and R^{10} are each hydrogen; and R^9 is



- In one more specific embodiment of such compounds of formula IC1, one of R^{11} and R^{13} is alkyl or cycloalkyl and the other is hydrogen. Examples of such claims include:
- 10 Penta-cyclo(Asp-Lys)-Asp-Achc-(D)Phe-Arg-Trp-Lys-NH₂; and Penta-cyclo(Asp-Lys)-Asp-Abc-(D) Phe-Arg-Trp-Lys-NH₂.

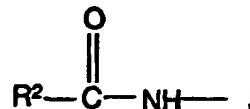
In another more specific embodiment of such compounds of formula IC1, one of R^{11} and R^{13} is phenyl and the other is hydrogen or phenyl. Examples of such compounds include Penta-cyclo(Asp-Lys)-Asp-4-Adpc-(D)Phe-Arg-Trp-Lys-NH₂.

- 15 In one embodiment of the compound of formula II, the compound of formula IIA, Z is



R^1 is

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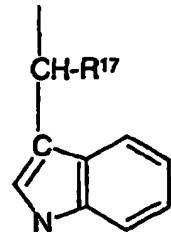


R^2 is alkyl;

$\text{R}^3, \text{R}^4, \text{R}^5, \text{R}^6$ and R^{10} are each hydrogen;

R^6 is hydrogen, halo, alkyl having from 1 to 3 carbon atoms, or alkoxy having from 1 to 3 carbon
5 atoms;

R^9 is



and R^{17} is as above.

In one embodiment of the compounds of formula II-A, as described in the preceding
10 paragraph, R^7 is NH. In a more specific embodiment, R^7 is NH and R^6 is hydrogen or alkyl.
Examples of such compounds include: Penta-cyclo(Asp-Lys)-Asp-(D,L)-Atc-(D)Phe-Arg-Trp-
Lys-NH₂; Penta-(Asp-Lys)-Asp-5-Me-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂; Penta-(Asp-Lys)-
Asp-5-Et-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂; and Penta-(Asp-Lys)-Asp-5-iPr-(D,L)Atc-
(D)Phe-Arg-Trp-Lys-NH₂.

15 In another specific embodiment of the compound of formula II-A, R^7 is NH and R^6 is halo.
Examples of such compounds include: Penta-(Asp-Lys)-Asp-5-BrAtc-(D)Phe-Arg-Trp-Lys-NH₂;
and Penta-(Asp-Lys)-Asp-5-ClAtc-(D)Phe-Arg-Trp-Lys-NH₂.

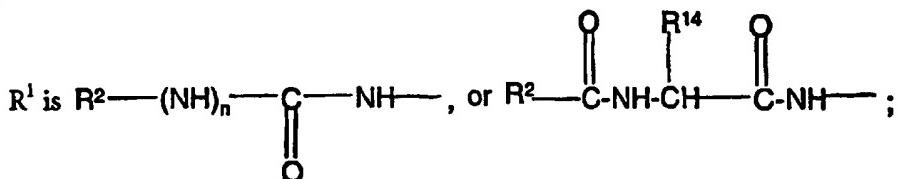
In another specific embodiment of the compound of formula II-A, R^7 is NH and R^6 is
alkoxy. Penta-(Asp-Lys)-Asp-5-MeO-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂; Penta-(Asp-Lys)-
20 Asp-5-EtO-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂; Penta-(Asp-Lys)-Asp-5-iPrO-(D,L)Atc-
(D)Phe-Arg-Trp-Lys-NH₂.

Another embodiment of the compounds of formula II are those compounds of formula II
in which Z, R^1 to R^5 and R^8 to R^{10} are as described above, R^7 is O and R^6 is halo. Examples of

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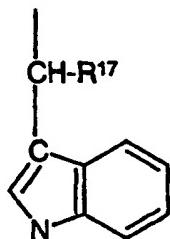
such compounds include: Penta-(Asp-Lys)-Asp-5-BrAtc-(D)Phe-Cit-Trp-Lys-NH₂; and Penta-(Asp-Lys)-Asp-5-ClAtc-(D)Phe-Cit-Trp-Lys-NH₂.

In another embodiment of the compound of formula II, the compound of formula II-B are
5 those compounds where Z is -S-S-;



R^3 , R^4 , R^5 , R^8 and R^{10} are each hydrogen; R^6 is hydrogen or halo; R^7 is NH; and

R^9 is



10 and R^{17} is as above.

Examples of such compounds of formula II-B include: Ac-Nle-cyclo(Cys-Cys)-Cys-(D,L)Atc-(D)Phe-Arg-Trp-Cys-NH₂; and Penta-cyclo(Cys-Cys)-Cys-5-Br(D,L)Atc-(D)Phe-Arg-Trp-Cys-NH₂.

This invention also provides the following compounds: Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Ala-Trp-Lys-NH₂; and Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Arg-(2S,3S) beta-methyl-Trp-Lys-NH₂.

The nomenclature used to define the peptides is that typically used in the art wherein the amino group at the N-terminus appears to the left and the carboxyl group at the C-terminus appears to the right. By natural amino acids is meant one of the naturally occurring amino acids found in proteins, i.e., Gly, Ala, Val, Leu, Ile, Ser, Thr, Lys, Arg, Asp, Asn, Glu, Gln, Cys, Met, Phe, Tyr, Pro, Trp, and His. Where the amino acid has isomeric forms, it is the L form of the amino acid that is represented unless otherwise explicitly indicated.

The following abbreviations or symbols are used to represent amino acids, protecting groups, solvents, reagents and the like.

	<u>Symbol</u>	<u>Meaning</u>
	β -Ala	beta-Alanine
	(2)-Nal	(2)-Naphthylalanine
	Atc	2-Aminotetraline-2-carboxylic acid
	5-BrAtc	5-Bromo-2-aminotetraline-2-carboxylic acid
10	5-ClAtc	5-Chloro-2-aminotetraline-2-carboxylic acid
	5-MeOAtc	5-Methoxy-2-aminotetraline-2-carboxylic acid
	5-EtOAtc	5-Ethoxy-2-aminotetraline-2-carboxylic acid
	5-iPrOAtc	5-Isopropoxy-2-aminotetraline-2-carboxylic acid
	5-MeAtc	5-Methyl-2-aminotetraline-2-carboxylic acid
15	5-EtAtc	5-Ethyl-2-aminotetraline-2-carboxylic acid
	5-iPrAtc	5-Isopropyl-2-aminotetraline-2-carboxylic acid
	5-DmaAtc	5-Dimethylamino-2-aminotetraline-2-carboxylic acid
	DBr	D-2,4-diaminobutanoic acid
	DPr	D-2,3-diaminopropionic acid
20	Sar	Sarcosine (N-methylglycine)
	Cit	Citrulline
	Apc	1-Amino-4-phenylcyclohexane-1-carboxylic acid
	4-HOApC	1-Amino-4-(4-hydroxyphenyl)cyclohexane-1-carboxylic acid

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	4-MeOApC	1-Amino-4-(4-methoxyphenyl)cyclohexane-1-carboxylic acid
	3-MeOApC	1-Amino-4-(4-methoxyphenyl)cyclohexane-1-carboxylic acid
	4-EtOApC	1-Amino-4-(4-ethoxyphenyl)cyclohexane-1-carboxylic acid
	4-iPrOApC	1-Amino-4-(4-isopropoxyphe ⁿ nyl)cyclohexane-1-carboxylic acid
5	4-MeApC	1-Amino-4-(4-methylphenyl)cyclohexane-1-carboxylic acid
	4-ClApC	1-Amino-4-(4-chlorophenyl)cyclohexane-1-carboxylic acid
	AppC	4-Amino-1-phenylpiperidine-4-carboxylic acid
	2-MeAppC	4-Amino-1-(2-methylphenyl)piperidine-4-carboxylic acid
	2-iProAppC	4-Amino-1-(2-isopropoxyphe ⁿ nyl)piperidine-4-carboxylic acid
10	3-MeAppC	4-Amino-1-(3-methylphenyl)piperidine-4-carboxylic acid
	3-MeOAppC	4-Amino-1-(3-methoxyphenyl)piperidine-4-carboxylic acid
	4-MeAppC	4-Amino-1-(4-methylphenyl)piperidine-4-carboxylic acid
	4-ClAppC	4-Amino-1-(4-chlorophenyl)piperidine-4-carboxylic acid
	4-PhOAppC	4-Amino-1-(4-phenoxyphenyl)piperidine-4-carboxylic acid
15	AchC	1-Amino-4-cyclohexylcyclohexane-1-carboxylic acid
	Adpc	1-Amino-4-diphenylcyclohexane-1-carboxylic acid
	Abc	1-Amino-4-tert-butylcyclohexane-1-carboxylic acid
	3-Amb	3-Aminomethyl benzoic acid
	4-Amb	4-Aminomethyl benzoic acid
20	2-Aba	2-Aminobenzoic acid
	Bu	Butyl
	Penta	pentanoyl
	Fmoc	9-Fluorenylmethoxycarbonyl

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Pmc	2,2,5,7,8-Pentamethylchroman-6-sulfonyl
Trt	Trityl (triphenylmethyl)
CH ₂ Cl ₂	Methylene chloride
CH ₃ CN	Acetonitrile
5 DMF	Dimethylformamide
DIPEA	N, N-Diisopropylethylamine
TFA	Trifluoroacetic acid
HOBT	N-Hydroxybenzotriazole
DIC	N, N'-Diisopropylcarbodiimide
10 BOP	Benzotriazol-1-yloxy-tris-(dimethylamino)phosphonium Hexafluorophosphate
PyBroP	Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate
HBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium Hexafluorophosphate
15 FAB-MS	Fast atom bombardment mass spectrometry
ES-MS	Electrospray mass spectrometry

Setting forth the substituted amino acid, in parentheses indicates analogs of the peptide sequence. Derivatization of the N-terminal amino group, is indicated to the left of the N-terminal substitution, separated by a hyphen. That is, for example, Ac-His-(D)Phe-Arg-Trp-Gly-NH₂ indicates a peptide having an amino acid sequence in which an acetyl group has been substituted for hydrogen at the N-terminus. The suffixes "-OH" and "-NH₂" following the hyphen or the parentheses refer to the free acid and amide forms of the polypeptide, respectively.

The linear peptides, used as the precursors for the present representative compounds may 25 be readily synthesized by any known conventional procedure for the formation of a peptide

linkage between amino acids. Such conventional procedures include, for example, any solution phase procedure permitting a condensation between the free alpha amino group of an amino acid or residue thereof having its carboxyl group or other reactive groups protected and the free primary carboxyl group of another amino acid or residue thereof having its amino group or other 5 reactive groups protected.

The process for synthesizing the linear peptides may be carried out by a procedure whereby each amino acid in the desired sequence is added one at a time in succession to another amino acid or residue thereof or by a procedure whereby peptide fragments with the desired amino acid sequence are first synthesized conventionally and then condensed to provide the desired peptide.

10 Such conventional procedures for synthesizing the precursor linear peptides include for example any solid phase peptide synthesis method. In such a method the synthesis of the novel compounds can be carried out by sequentially incorporating the desired amino acid residues one at a time into the growing peptide chain according to the general principles of solid phase methods [Merrifield, R. B., *J. Amer. Chem. Soc.* 1963, 85, 2149-2154; Barany *et al.*, *The peptides, Analysis, Synthesis and Biology*, Vol. 2, Gross, E. and Meienhofer, J., Eds. Academic Press 1-284 15 (1980)].

Common to chemical syntheses of peptides is the protection of reactive side chain groups of the various amino acid moieties with suitable protecting groups, which will prevent a chemical reaction from occurring at that site until the protecting group is ultimately removed. Usually 20 also common is the protection of the alpha amino group of an amino acid or fragment while that entity reacts at the carboxyl group, followed by the selective removal of the alpha amino protecting group and allow a subsequent reaction to take place at that site. While specific protecting groups have been disclosed in regard to the solid phase synthesis method, it should be noted that each amino acid can be protected by an protective group conventionally used for the 25 respective amino acid in solution phase synthesis.

Alpha amino groups may be protected by a suitable protecting group selected from aromatic urethane-type protecting groups, such as benzyloxycarbonyl (Z) and substituted benzyloxycarbonyl, such as *p*-chlorobenzyloxycarbonyl, *p*-nitrobenzyloxycarbonyl, *p*-bromobenzyloxycarbonyl, *p*-biphenyl-isopropoxycarbonyl, 9-fluorenylmethoxycarbonyl (Fmoc) 30 and *p*-methoxybenzyloxycarbonyl (Mo2); aliphatic urethane-type protecting groups, such as *t*-butyloxycarbonyl (Boc), diisopropylmethoxycarbonyl, isopropoxycarbonyl, and allyloxycarbonyl. Herein, Fmoc is the most preferred for alpha amino protection.

Guanidino groups may be protected by a suitable protecting group selected from nitro, p-toluenesulfonyl (Tos), Z, pentamethylchromanesulfonyl (Pmc), adamantlyloxycarbonyl, and Boc. Pmc is the most preferred for arginine (Arg).

All solvents, isopropanol (iPrOH), methylene chloride (CH_2Cl_2), dimethylformamide (DMF) and N-methylpyrrolidinone (NMP) were purchased from Fisher or Burdick & Jackson and were used without additional distillation. Trifluoroacetic acid was purchased from Halocarbon or Fluka and used without further purification. Diisopropylcarbodiimide (DIC) and diisopropylethylamine (DIPEA) was purchased from Fluka or Aldrich and used without further purification. Hydroxybenzotriazole (HOBT) dimethylsulfide (DMS) and 1,2-ethanedithiol (EDT) were purchased from Sigma Chemical Co. and used without further purification. Protected amino acids were generally of the L configuration and were obtained commercially from Bachem, Advanced ChemTech, or Neosystem. Purity of these reagents was confirmed by thin layer chromatography, NMR and melting point prior to use. Benzhydrylamine resin (BHA) was a copolymer of styrene - 1% divinylbenzene (100-200 or 200-400 mesh) obtained from Bachem or Advanced Chemtech. Total nitrogen content of these resins were generally between 0.3 - 1.2 meq/g.

High performance liquid chromatography (HPLC) was conducted on a LDC apparatus consisting of Constametric I and III pumps, a Gradient Master solvent programmer and mixer, and a Spectromonitor III variable wavelength UV detector. Analytical HPLC was performed in reversed phase mode using Vydac C₁₈ columns (0.4 x 30 cm). Preparative HPLC separations were run on Vydac columns (2 x 25 cm).

The linear peptides were preferably prepared using solid phase synthesis by the method generally described by Merrifield, [J. Amer. Chem. Soc., 1963, 85, 2149], although other equivalent chemical synthesis known in the art could be used as previously mentioned. Solid phase synthesis is commenced from the C-terminal end of the peptide by coupling a protected alpha-amino acid to a suitable resin. Such a starting material can be prepared by attaching an alpha-amino-protected amino acid by an ester linkage to a p-benzyloxybenzyl alcohol (Wang) resin, or by an amide bond between an Fmoc-Linker, such as p-[(R, S)- α -[1-(9H-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl]-phenoxyacetic acid (Rink linker) to a benzhydrylamine (BHA) resin. Preparation of the hydroxymethyl resin is well known in the art. Fmoc-Linker-BHA resin supports are commercially available and generally used when the desired peptide being synthesized has an unsubstituted amide at the C-terminus.

Since the compounds of the present invention are cyclic peptides, prepared by the formation of a lactam or a disulfide bond, the precursor linear peptides are assembled so as to

place suitable amino acids or mimetics carrying the appropriate side chain residues in positions in the linear peptides that can ultimately be induced to undergo an intramolecular amide bond or disulfide bond formation. The lactams are formed through the coupling a side chain amino functionality of a C-terminal amino acid residue with a distal carboxylic acid residue, while the 5 disulfide bond is formed through the oxidative coupling of two cysteine residues appropriately incorporated at the C-terminal and at or near the N-terminus of the precursor linear peptide. For example, in the preparation of the lactam peptides, in the precursor linear pentapeptides the N-cap can be used as a template to incorporate a carboxyl residue, e.g. structure X, or in the case of hexapeptides, the peptide is designed such that the N-terminal amino acid residue is chosen 10 from one of the amino acids containing a suitably protected side chain carboxylic acid group, eg aspartic acid, glutamic acid. In the linear heptapeptides, either aspartic acid or glutamic acid are incorporated as the penultimate residue from the N-terminus. In all the precursor linear peptides, i.e., the hepta- hexa- and pentapeptides, the C-terminal residue is chosen from natural or unnatural amino acid bearing a suitably protected basic side chain residue, capable of forming 15 a amide bond when deprotected, for example lysine, ornithine, 2,3-diaminopropanoic acid, 2,4-diaminobutanoic acid. To form a cyclic peptide containing a disulfide bond, where the precursor is a linear hexapeptide, the peptide is designed such that an appropriately S-protected cysteine residue is incorporated as both the C and N-terminal residues, while if the precursor is a linear heptapeptide the appropriately S-protected cysteine residues are incorporated as both the C 20 terminal residue and the penultimate N-terminal residue as in for example X.

In general, to prepare the linear peptides, the amino acids or mimetic are coupled onto the Fmoc-Linker-BHA resin using the Fmoc protected form of amino acid or mimetic, with 2 - 5 equivalents of amino acid and a suitable coupling reagent. After couplings, the resin may be washed and dried under vacuum. Loading of the amino acid onto the resin may be determined 25 by amino acid analysis of an aliquot of Fmoc-amino acid resin or by determination of Fmoc groups by UV analysis. Any unreacted amino groups may be capped by reacting the resin with acetic anhydride and diisopropylethylamine in methylene chloride.

The resins are carried through several repetitive cycles to add amino acids sequentially. The alpha amino Fmoc protecting groups are removed under basic conditions. Piperidine, 30 piperazine or morpholine (20-40% v/v) in DMF may be used for this purpose. Preferably 40% piperidine in DMF is utilized.

Following the removal of the alpha amino protecting group, the subsequent protected amino acids are coupled stepwise in the desired order to obtain an intermediate, protected peptide-resin. The activating reagents used for coupling of the amino acids in the solid phase 35 synthesis of the peptides are well known in the art. For example, appropriate reagents for such

syntheses are benzotriazol-1-yloxy-tri-(dimethylamino) phosphonium hexafluorophosphate (BOP), Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP), 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), and diisopropylcarbodiimide (DIC). Preferred here are HBTU and DIC. Other activating agents as described by Barany and Merrifield [The Peptides, Vol. 2, J. Meienhofer, ed., Academic Press, 1979, pp 1-284] may be utilized. Various reagents such as 1-hydroxybenzotriazole (HOBT), N-hydroxysuccinimide (HOSu) and 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (HOOBT) may be added to the coupling mixtures in order to optimize the synthetic cycles. Preferred here is HOBT.

10 The protocol for a typical synthetic cycle is as follows:

Protocol 1

	<u>Step</u>	<u>Reagent</u>	<u>Time</u>
	1 DMF	2 x 30 sec	
15	2 40% piperidine/DMF		1 min
	3 40% piperidine/DMF		15 min
	4	DMF	2 x 30 sec
	5 iPrOH	2 x 30 sec	
	6 DMF	3 x 30 sec	
20	7 coupling	60 min - 18 hours	
	8 DMF	2 x 30 sec	
	9 iPrOH	1 x 30 sec	
	10	DMF	1 x 30 sec
	11	CH ₂ Cl ₂	2 x 30 sec

25 Solvents for all washings and couplings were measured to volumes of 10 - 20 ml/g resins. Coupling reactions throughout the synthesis were monitored by the Kaiser ninhydrin test to

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determine extent of completion [Kaiser *et al.* *Anal. Biochem.* 1970, 34, 595-598]. Slow reaction kinetics was observed for Fmoc-Arg (Pmc) and for couplings to secondary amines by sterically hindered acids. Any incomplete coupling reactions were either recoupled with freshly prepared activated amino acid or capped by treating the peptide resin with acetic anhydride as described above. The fully assembled peptide-resins were dried in vacuum for several hours.

For each compound, the blocking groups were removed and the linear peptide cleaved from the resin by the following procedure. Generally, The peptide-resins are treated with 100 μ L ethanedithiol, 100 μ L dimethylsulfide, 300 μ L anisole, and 9.5 mL trifluoroacetic acid, per gram of resin, at room temperature for 120 min. The resin is filtered off and the filtrates are precipitated in chilled ethyl ether. The precipitates are centrifuged and the ether layer is decanted. The residue is washed with two or three volumes of Et₂O and recentrifuged. If desired, the crude linear peptides are purified by preparative HPLC. The peptides were applied to the columns in a minimum volume of either AcOH/H₂O or 0.1% TFA/H₂O. Gradient elution is generally started at 10% B buffer, 10% -60% B in 90 minutes, (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) at a flow rate of 8 mL/min. UV detection is made at 280 nm. Fractions were collected at 1.0 - 2.5 minute intervals and inspected by analytical HPLC. Fractions judged to be of high purity were pooled and lyophilized.

To prepare the lactams, the appropriate unpurified linear peptide is dissolved in a suitable inert solvent, for example N-methyl pyrrolidone or DMF, preferably DMF and adjusted to an apparent pH of 8.0 by the addition of a tertiary amine base, for example N-methyl morpholine, and then is treated with an amide bond forming reagent, preferably BOP. The reaction is conveniently carried out at a temperature between 40 °C and 0 °C, preferably at about room temperature. Purification of the crude cyclic peptides is carried out by preparative HPLC. Gradient elution is generally started at 20% B buffer, 20% -60% B in 90 minutes, (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) at a flow rate of 8 mL/min. UV detection is made at 280 nm. Fractions were collected and monitored by analytical HPLC. Fractions judged to be of high purity were pooled and lyophilized.

To prepare the cyclic disulfide peptides, the purified HPLC-purified linear peptide containing two appropriately positioned cysteine residues, is dissolved at a fairly high dilution level in an suitable inert solvent mixture, for example aqueous DMSO and the solution is adjusted to pH 8.0 by the careful addition of ammonium hydroxide. Oxygen is then bubbled into the stirred solution. The reaction is conveniently carried out at a temperature between 40 °C and 0 °C, preferably at about room temperature and the progress of the cyclization is monitored by analytical HPLC. After the reaction is judged to be completed, the solution is lyophilized and

the crude cyclic peptide is purified by preparative HPLC. Gradient elution is generally started at 20% B buffer, 20% -60% B in 90 minutes, (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) at a flow rate of 8 mL/min. UV detection is made at 280 nm. Fractions were collected and monitored by analytical HPLC. Fractions judged to be of high purity were pooled and lyophilized.

Purification of the crude peptides is carried out by preparative HPLC. The peptides were applied to the columns in a minimum volume of either AcOH/H₂O or 0.1% TFA/H₂O. Gradient elution is generally started at 10% B buffer, 10% -60% B in 90 minutes, (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) at a flow rate of 8 mL/min. UV detection is made at 280 nm. Fractions were collected at 1.0 - 2.5 minute intervals and inspected by analytical HPLC. Fractions judged to be of high purity were pooled and lyophilized.

Purity of the final products is checked by analytical HPLC on a reversed phase column as stated above. Purity of all products is judged to be approximately 95 - 99%. All final products were also subjected to fast atom bombardment mass spectrometry (FAB-MS) or electrospray mass spectrometry (ES-MS). All products yielded the expected parent M+H ions within acceptable limits.

The process for synthesizing the representative compounds may be carried out by a procedure whereby each amino acid in the desired sequence is added one at a time in succession to another amino acid or residue thereof or by a procedure whereby peptide fragments with the desired amino acid sequence are first synthesized conventionally and then condensed to provide the desired peptide.

Such conventional procedures for synthesizing the novel compounds of the present invention include for example any solid phase peptide synthesis method. In such a method the synthesis of the novel compounds can be carried out by sequentially incorporating the desired amino acid residues one at a time into the growing peptide chain according to the general principles of solid phase methods [Merrifield, R. B., *J. Amer. Chem. Soc.* 1963, 85, 2149-2154; Barany *et al.*, *The peptides, Analysis, Synthesis and Biology*, Vol. 2, Gross, E. and Meienhofer, J., Eds. Academic Press 1-284 (1980)].

Common to chemical syntheses of peptides is the protection of reactive side chain groups of the various amino acid moieties with suitable protecting groups, which will prevent a chemical reaction from occurring at that site until the protecting group is ultimately removed. Usually also common is the protection of the alpha amino group of an amino acid or fragment while that entity reacts at the carboxyl group, followed by the selective removal of the alpha amino

protecting group and allow a subsequent reaction to take place at that site. While specific protecting groups have been disclosed in regard to the solid phase synthesis method, it should be noted that each amino acid can be protected by an protective group conventionally used for the respective amino acid in solution phase synthesis.

- 5 Alpha amino groups may be protected by a suitable protecting group selected from aromatic urethane-type protecting groups, such as benzyloxycarbonyl (Z) and substituted benzyloxycarbonyl, such as *p*-chlorobenzyloxycarbonyl, *p*-nitrobenzyloxycarbonyl, *p*-bromobenzyloxycarbonyl, *p*-biphenyl-isopropoxycarbonyl, 9-fluorenylmethoxycarbonyl (Fmoc) and *p*-methoxybenzyloxycarbonyl (Moz); aliphatic urethane-type protecting groups, such as *t*-butyloxycarbonyl (Boc), diisopropylmethoxycarbonyl, isopropoxycarbonyl, and allyloxycarbonyl. Herein, Fmoc is the most preferred for alpha amino protection.
- 10

Guanidino groups may be protected by a suitable protecting group selected from nitro, *p*-toluenesulfonyl (Tos), Z, pentamethylchromanesulfonyl (Pmc), adamantlyloxycarbonyl, and Boc. Pmc is the most preferred for arginine (Arg).

- 15 All solvents, isopropanol (iPrOH), methylene chloride (CH_2Cl_2), dimethylformamide (DMF) and N-methylpyrrolidinone (NMP) were purchased from Fisher or Burdick & Jackson and were used without additional distillation. Trifluoroacetic acid was purchased from Halocarbon or Fluka and used without further purification. Diisopropylcarbodiimide (DIC) and diisopropylethylamine (DIPEA) was purchased from Fluka or Aldrich and used without further purification. Hydroxybenzotriazole (HOBT) dimethylsulfide (DMS) and 1,2-ethanedithiol (EDT) were purchased from Sigma Chemical Co. and used without further purification. Protected amino acids were generally of the L configuration and were obtained commercially from Bachem, Advanced ChemTech, or Neosystem. Purity of these reagents was confirmed by thin layer chromatography, NMR and melting point prior to use. Benzhydrylamine resin (BHA)
- 20
- 25 was a copolymer of styrene - 1% divinylbenzene (100-200 or 200-400 mesh) obtained from Bachem or Advanced Chemtech. Total nitrogen content of these resins were generally between 0.3 - 1.2 meq/g.

- 30 High performance liquid chromatography (HPLC) was conducted on a LDC apparatus consisting of Constametric I and III pumps, a Gradient Master solvent programmer and mixer, and a Spectromonitor III variable wavelength UV detector. Analytical HPLC was performed in reversed phase mode using Vydac C₁₈ columns (0.4 x 30 cm). Preparative HPLC separations were run on Vydac columns (2 x 25 cm).

Peptides were preferably prepared using solid phase synthesis by the method generally described by Merrifield, [J. Amer. Chem. Soc., 1963, 85, 2149], although other equivalent chemical synthesis known in the art could be used as previously mentioned. Solid phase synthesis is commenced from the C-terminal end of the peptide by coupling a protected alpha-
5 amino acid to a suitable resin. Such a starting material can be prepared by attaching an alpha-amino-protected amino acid by an ester linkage to a p-benzyloxybenzyl alcohol (Wang) resin, or by an amide bond between an Fmoc-Linker, such as p-[(R, S)- α -[1-(9H-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl]-phenoxyacetic acid (Rink linker) to a benzhydrylamine (BHA) resin. Preparation of the hydroxymethyl resin is well known in the art.
10 Fmoc-Linker-BHA resin supports are commercially available and generally used when the desired peptide being synthesized has an unsubstituted amide at the C-terminus.

In general, the amino acids or mimetic are coupled onto the Fmoc-Linker-BHA resin using the Fmoc protected form of amino acid or mimetic, with 2 - 5 equivalents of amino acid and a suitable coupling reagent. After couplings, the resin may be washed and dried under vacuum.
15 Loading of the amino acid onto the resin may be determined by amino acid analysis of an aliquot of Fmoc-amino acid resin or by determination of Fmoc groups by UV analysis. Any unreacted amino groups may be capped by reacting the resin with acetic anhydride and diisopropylethylamine in methylene chloride.

The resins are carried through several repetitive cycles to add amino acids sequentially.
20 The alpha amino Fmoc protecting groups are removed under basic conditions. Piperidine, piperazine or morpholine (20-40% v/v) in DMF may be used for this purpose. Preferably 40% piperidine in DMF is utilized.

Following the removal of the alpha amino protecting group, the subsequent protected amino acids are coupled stepwise in the desired order to obtain an intermediate, protected
25 peptide-resin. The activating reagents used for coupling of the amino acids in the solid phase synthesis of the peptides are well known in the art. For example, appropriate reagents for such syntheses are benzotriazol-1-yloxy-tri-(dimethylamino) phosphonium hexafluorophosphate (BOP), Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP), 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), and
30 diisopropylcarbodiimide (DIC). Preferred here are HBTU and DIC. Other activating agents as described by Barany and Merrifield [The Peptides, Vol. 2, J. Meienhofer, ed., Academic Press, 1979, pp 1-284] may be utilized. Various reagents such as 1-hydroxybenzotriazole (HOBT), N-hydroxysuccinimide (HOSu) and 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (HOOBT)

- 29 -

may be added to the coupling mixtures in order to optimize the synthetic cycles. Preferred here is HOBT.

The protocol for a typical synthetic cycle is as follows:

Protocol 1

	<u>Step</u>	<u>Reagent</u>	<u>Time</u>
5	1 DMF	2 x 30 sec	
	2 40% piperidine/DMF		1 min
	3 40% piperidine/DMF		15 min
	4	DMF	2 x 30 sec
10	5 iPrOH	2 x 30 sec	
	6 DMF	3 x 30 sec	
	7 coupling		60 min - 18 hours
	8 DMF	2 x 30 sec	
	9 iPrOH	1 x 30 sec	
15	10	DMF	1 x 30 sec
	11	CH ₂ Cl ₂	2 x 30 sec

Solvents for all washings and couplings were measured to volumes of 10 - 20 ml/g resins. Coupling reactions throughout the synthesis were monitored by the Kaiser ninhydrin test to determine extent of completion [Kaiser *et al. Anal. Biochem.* 1970, 34, 595-598]. Slow reaction kinetics was observed for Fmoc-Arg (Pmc) and for couplings to secondary amines by sterically hindered acids. Any incomplete coupling reactions were either recoupled with freshly prepared activated amino acid or capped by treating the peptide resin with acetic anhydride as described above. The fully assembled peptide-resins were dried in vacuum for several hours.

For each compound, the blocking groups were removed and the peptide cleaved from the resin by the following procedure. The peptide-resins were treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 300 µL anisole, and 9.5 mL trifluoroacetic acid, per gram of resin, at room

- 30 -

temperature for 120 min. The resin is filtered off and the filtrates are precipitated in chilled ethyl ether. The precipitates are centrifuged and the ether layer is decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged. The crude products are dried under vacuum.

5

Purification of Crude Peptide Preparations

Purification of the crude peptides was carried out by preparative HPLC. The peptides were applied to the columns in a minimum volume of either AcOH/H₂O or 0.1% TFA/H₂O. Gradient elution was generally started at 10% B buffer, 10% -60% B in 90 minutes, (buffer A: 10 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) at a flow rate of 8 mL/min. UV detection was made at 280 nm. Fractions were collected at 1.0 - 2.5 minute intervals and inspected by analytical HPLC. Fractions judged to be of high purity were pooled and lyophilized.

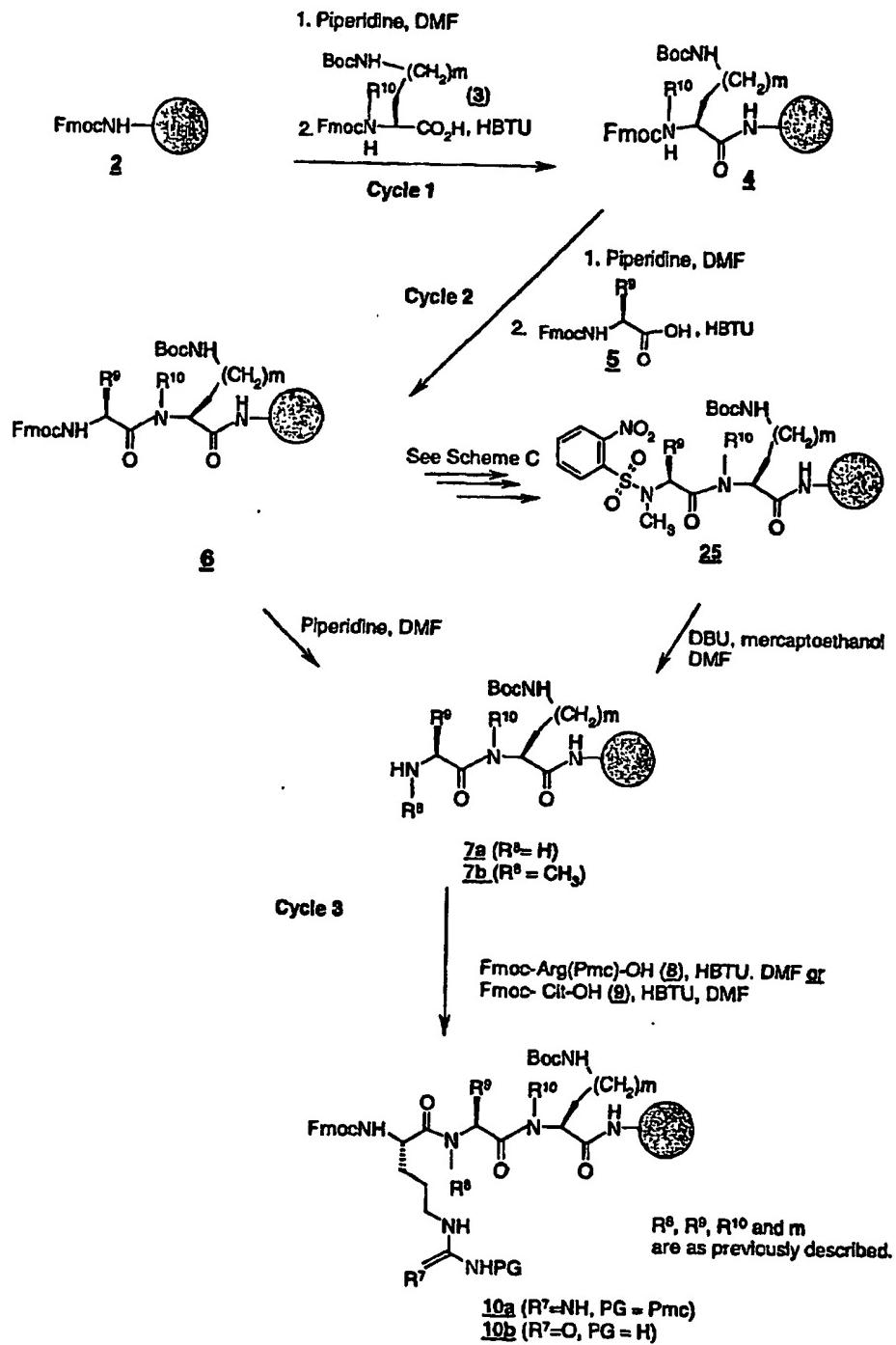
Purity of the final products was checked by analytical HPLC on a reversed phase column as stated above. Purity of all products was judged to be approximately 95 - 99%. All final products 15 were also subjected to fast atom bombardment mass spectrometry (FAB-MS) or electrospray mass spectrometry (ES-MS). All products yielded the expected parent M+H ions within acceptable limits.

Utilizing the techniques described above, the compounds of this invention can be synthesized in accordance with the following reaction schemes.

20

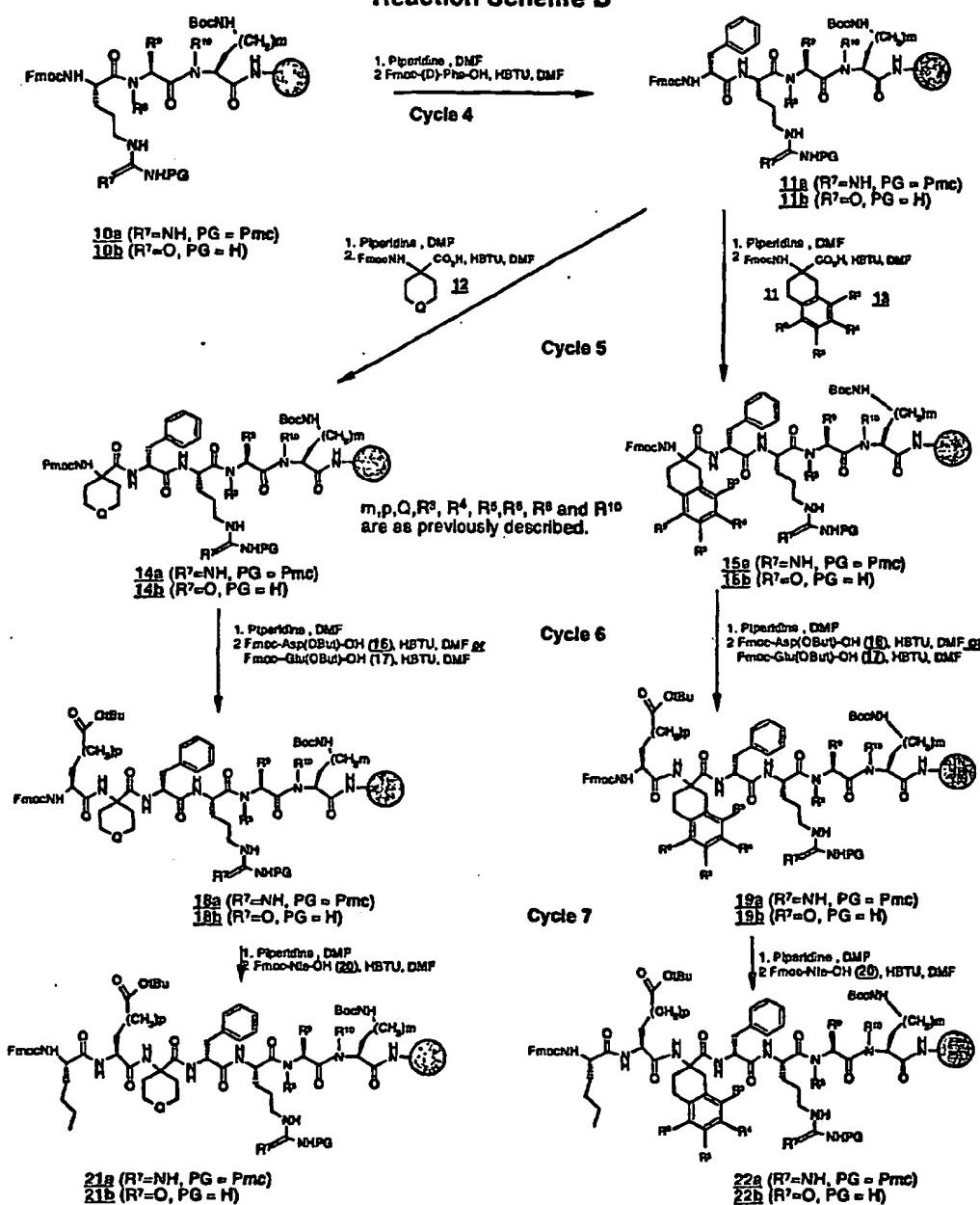
- 31 -

Reaction Scheme A



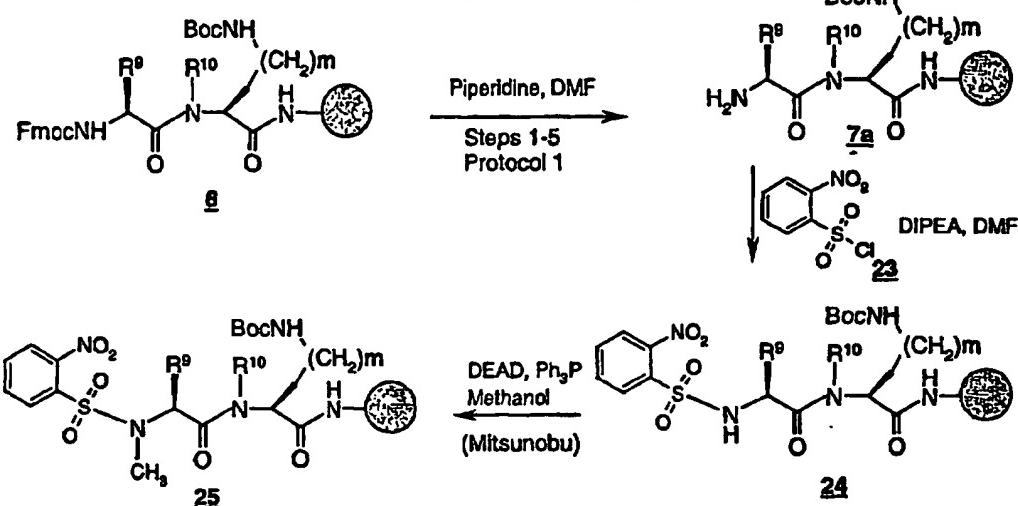
- 32 -

Reaction Scheme B



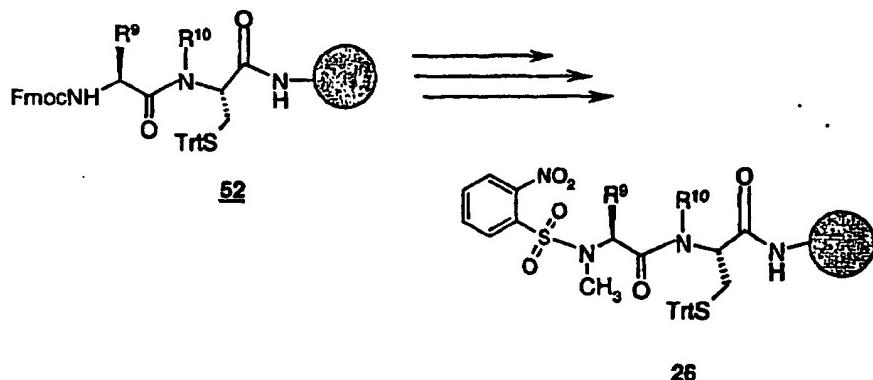
- 33 -

Reaction Scheme C



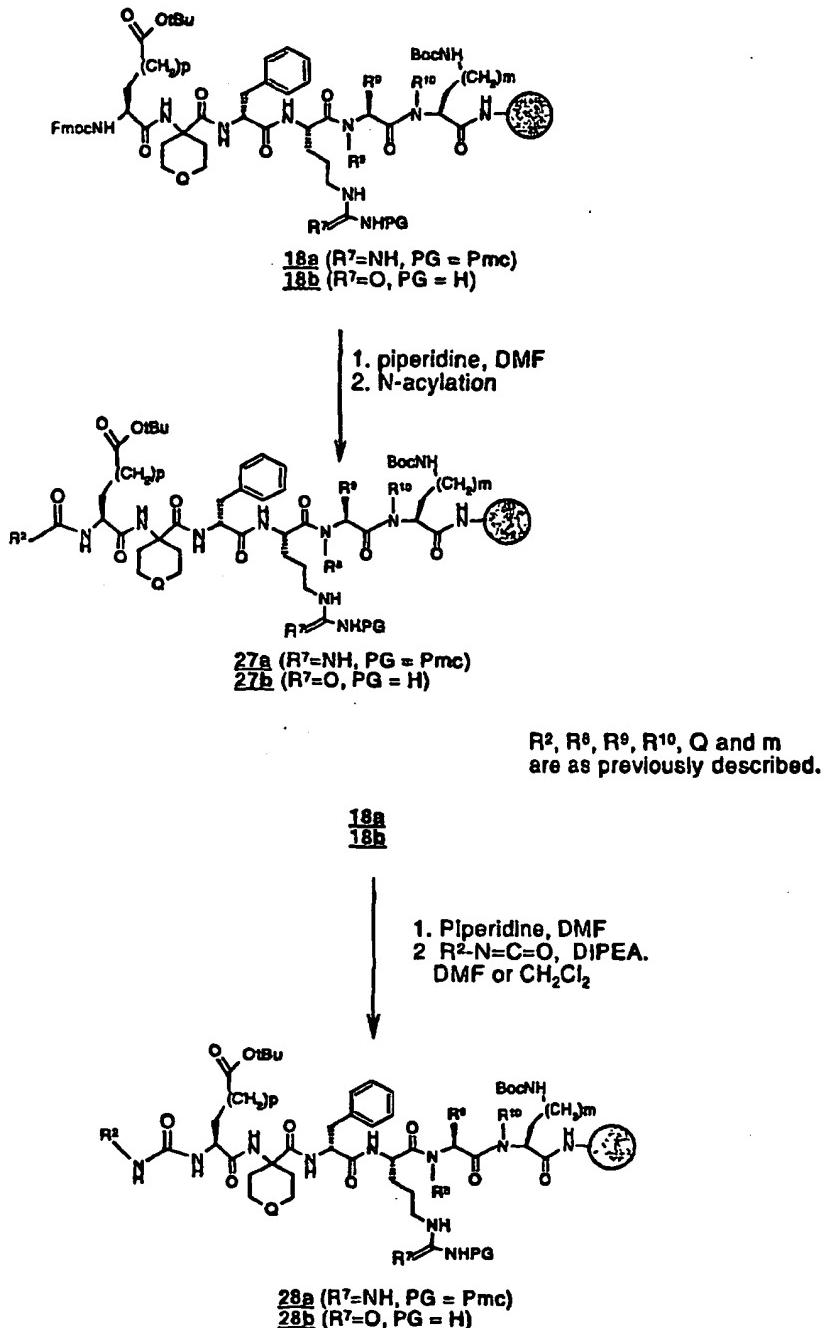
R^9 , R^{10} , and m
are as previously described.

In the same manner:



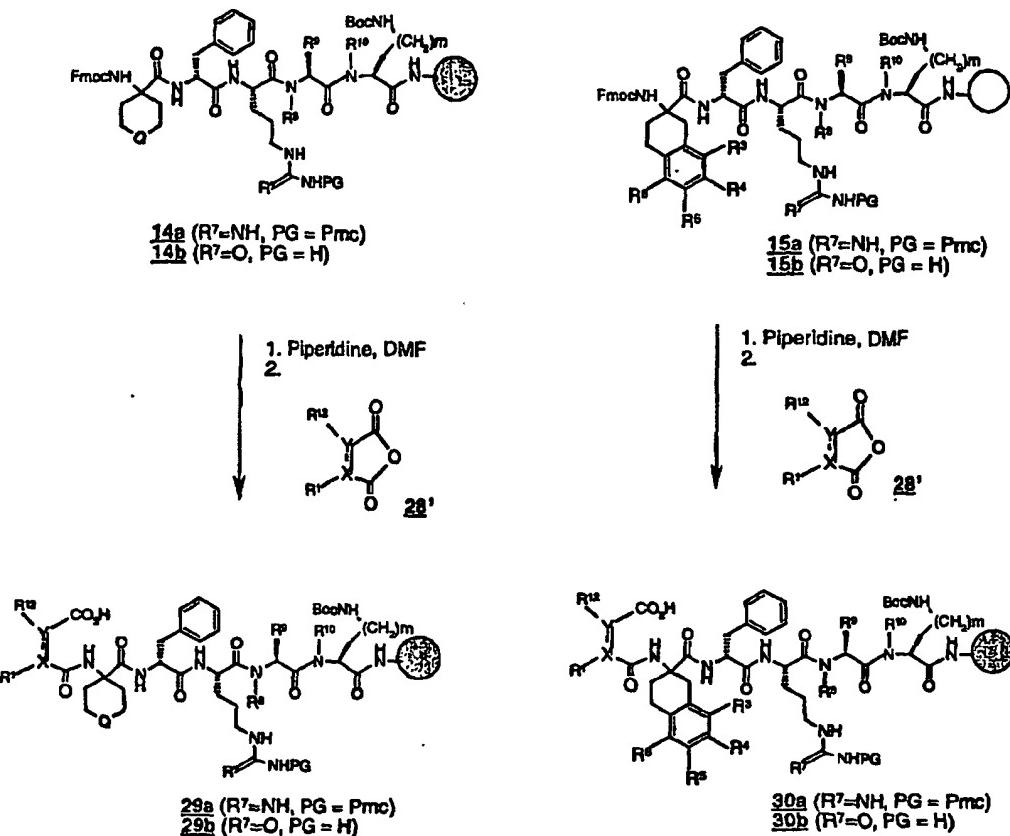
- 34 -

Reaction Scheme D



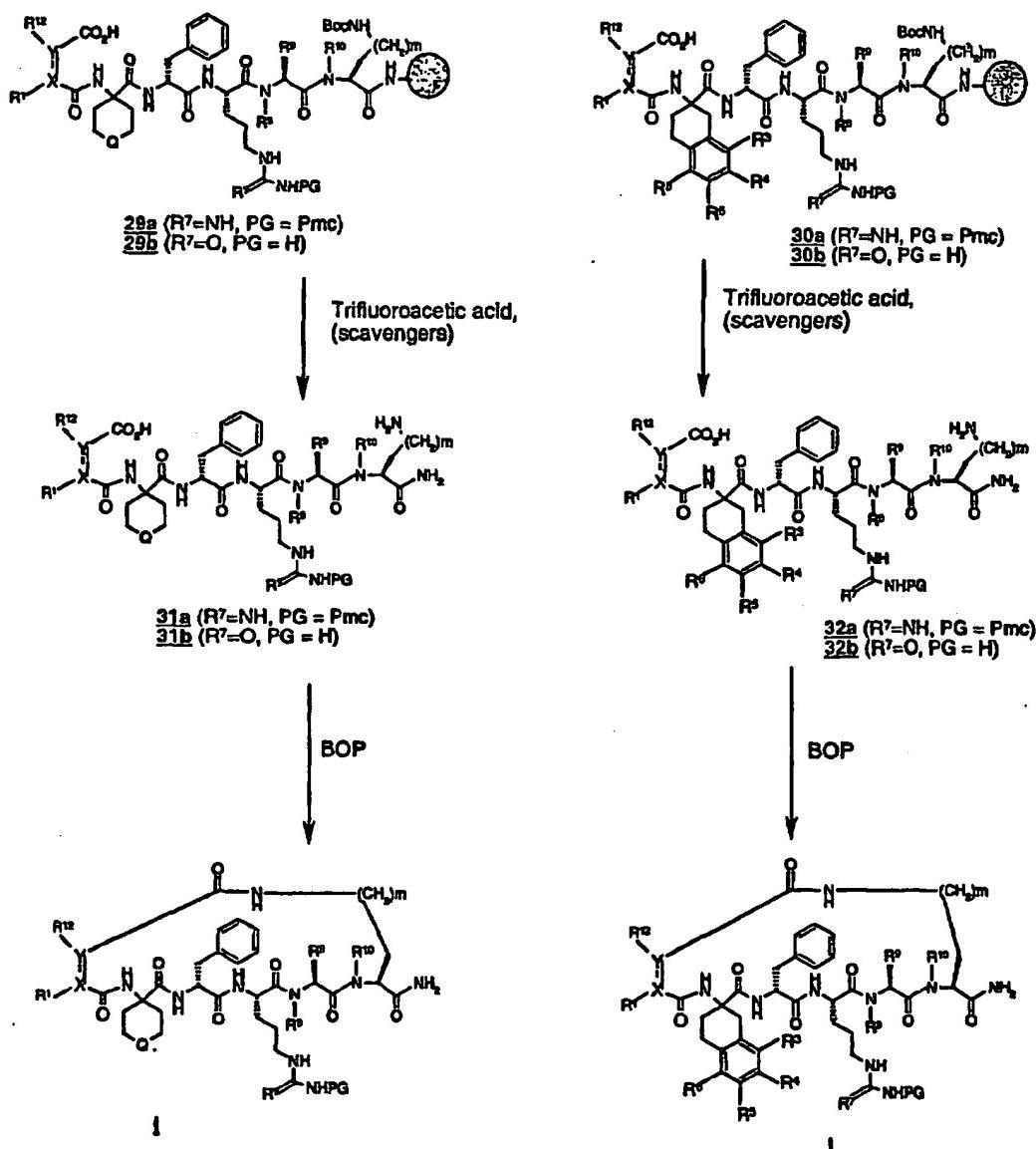
- 35 -

Reaction Scheme F

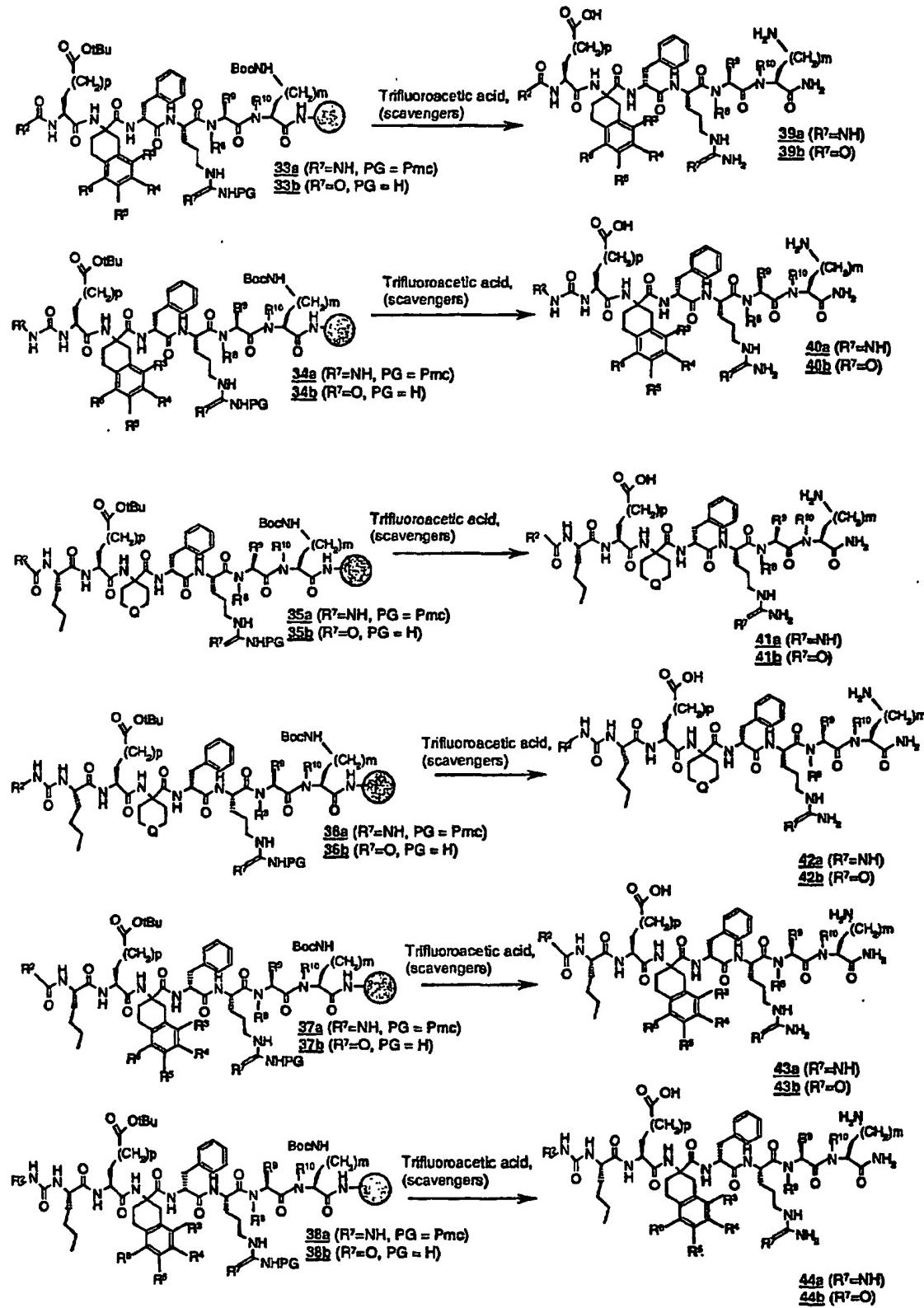


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Reaction Scheme G

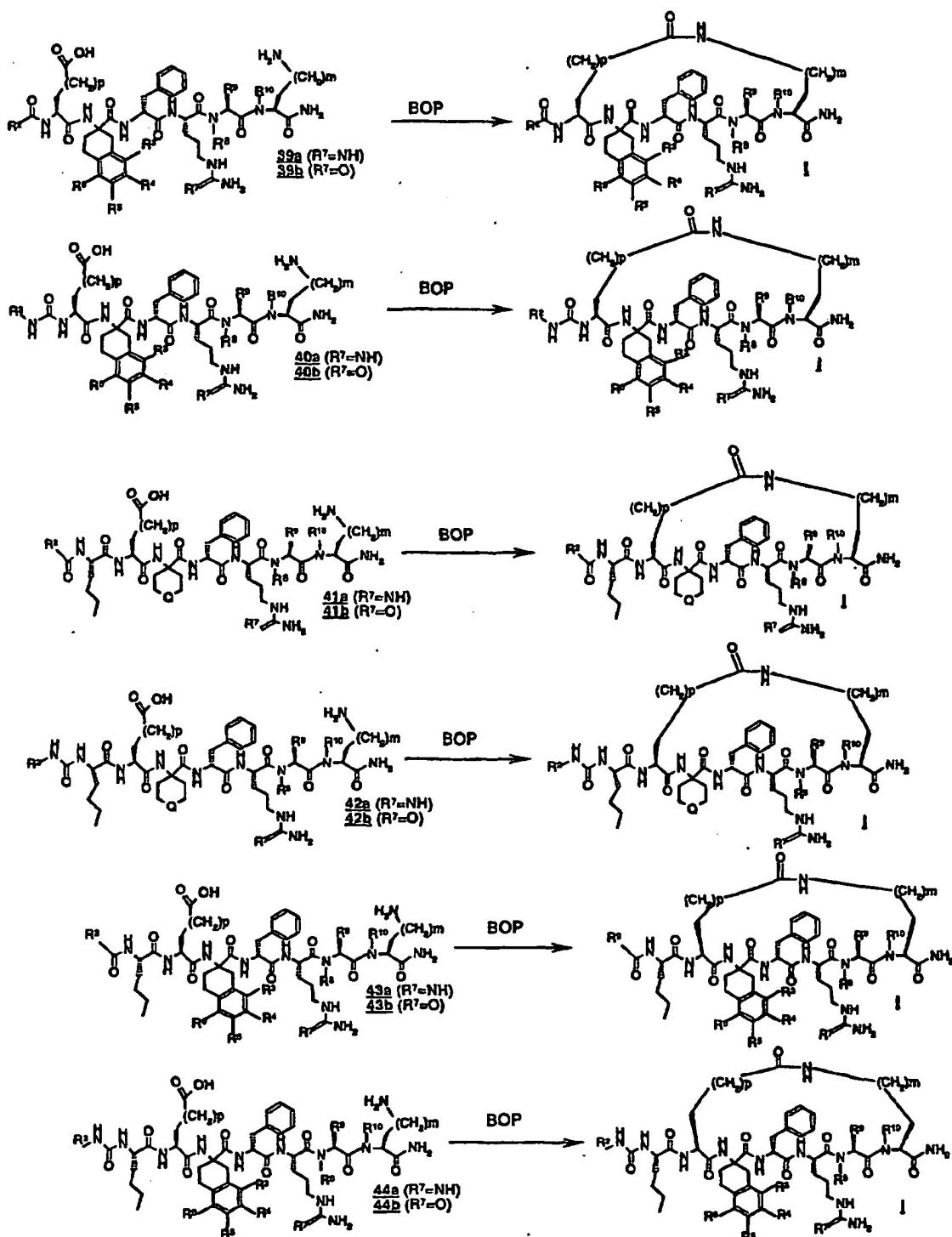


Reaction Scheme H



- 38 -

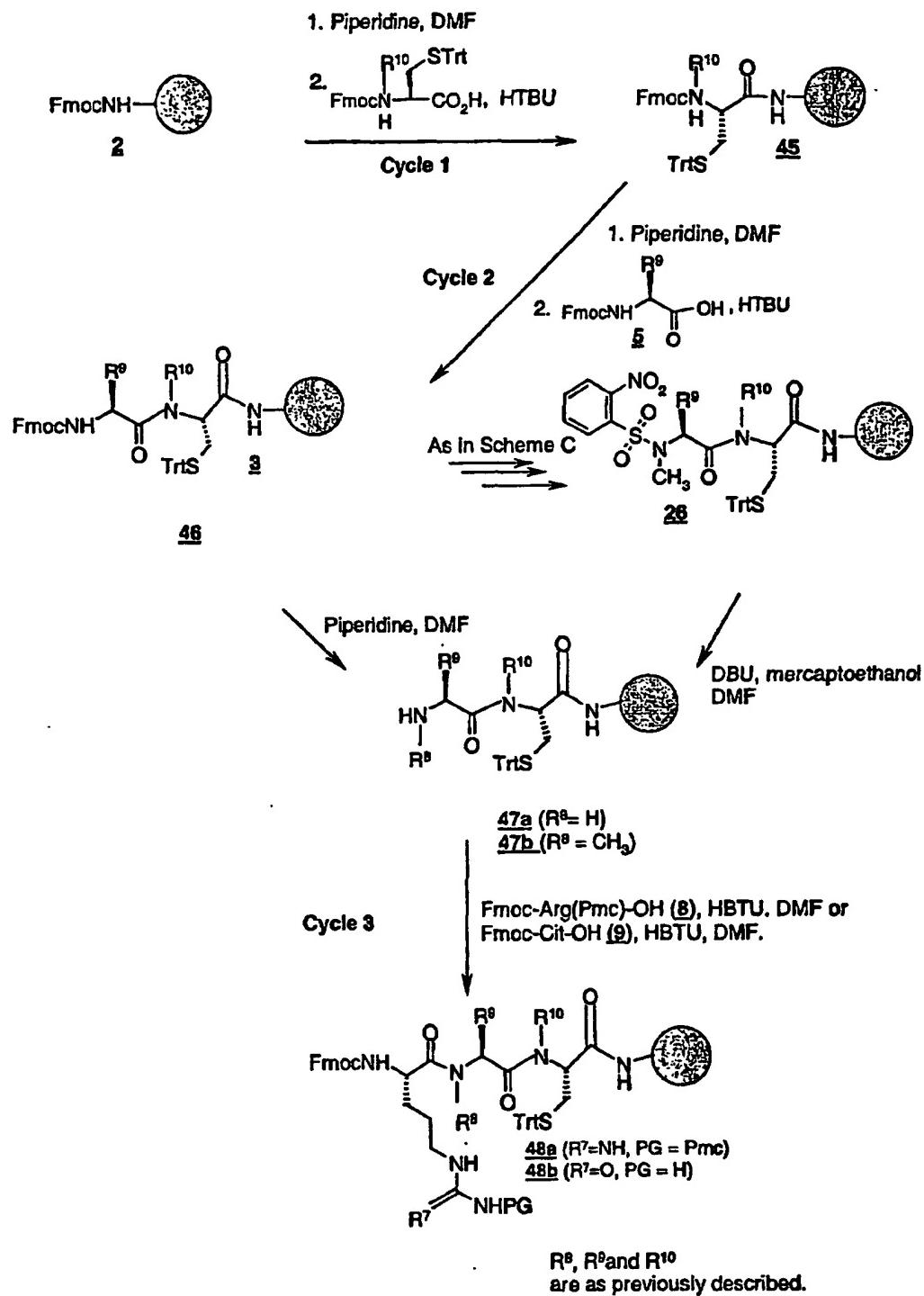
Reaction Scheme I



$R^2, R^3, R^4, R^5, R^6, R^8, R^9, R^{10}, O, p$, and m are as previously described

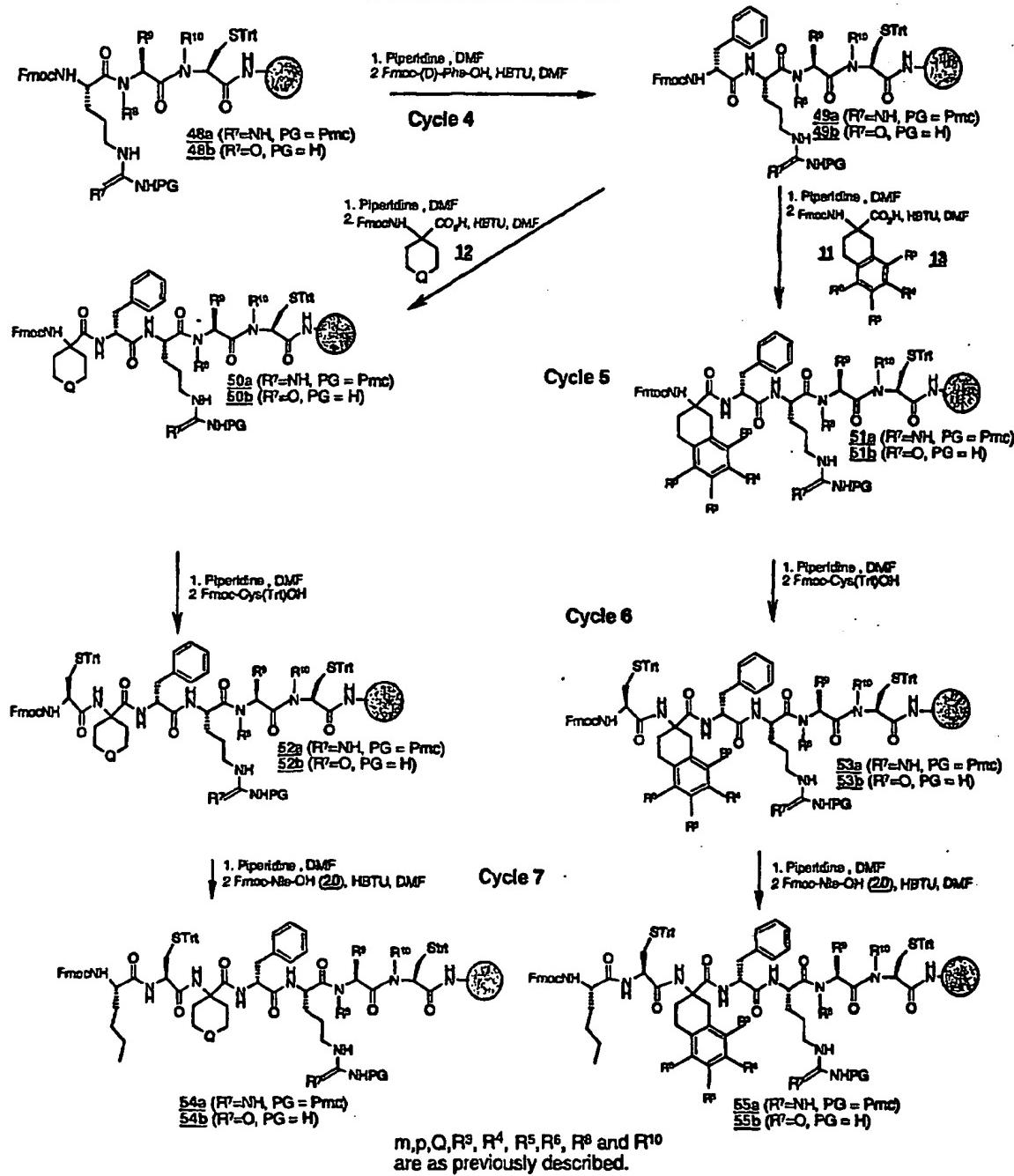
- 39 -

Reaction Scheme J



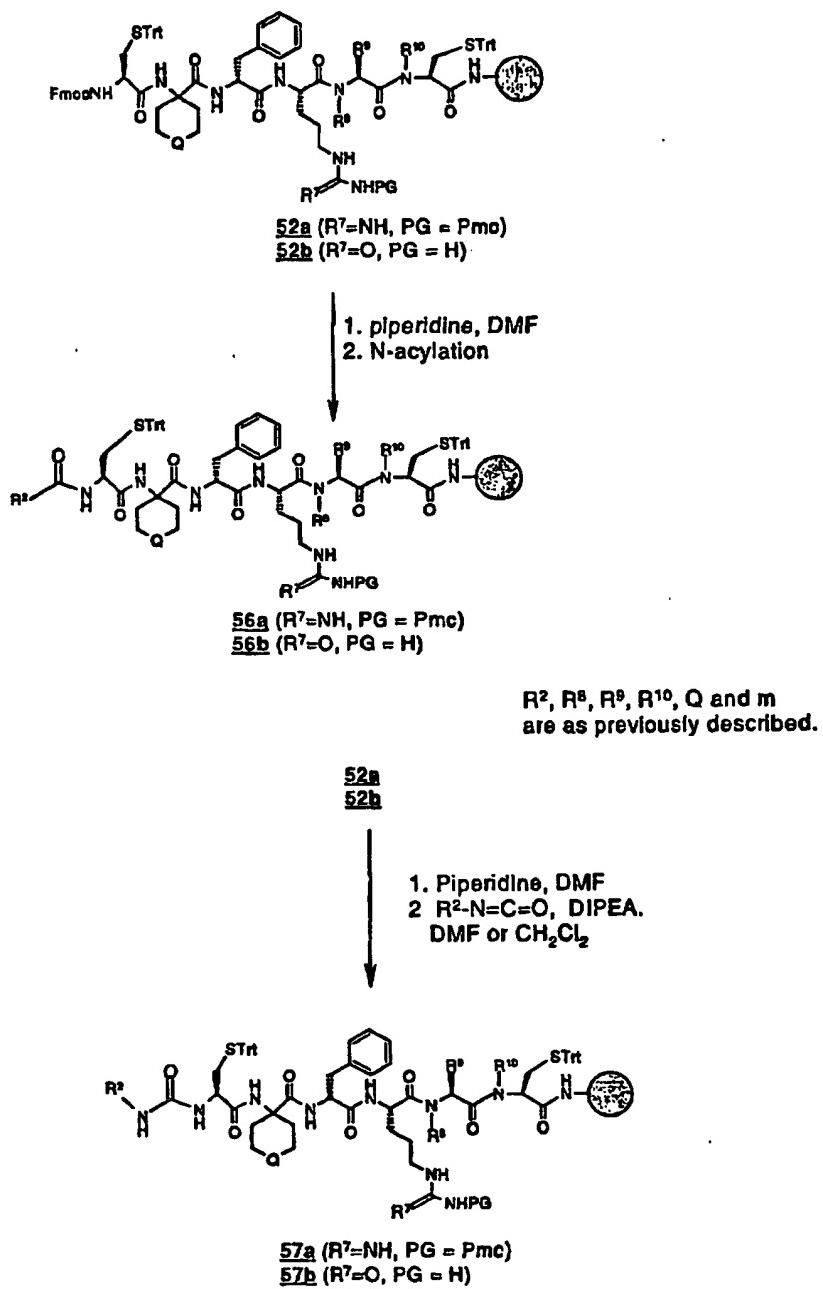
- 40 -

Reaction Scheme K



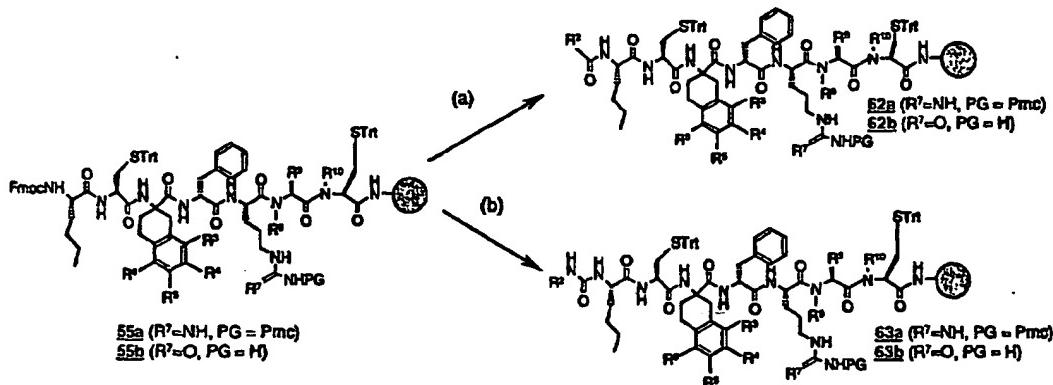
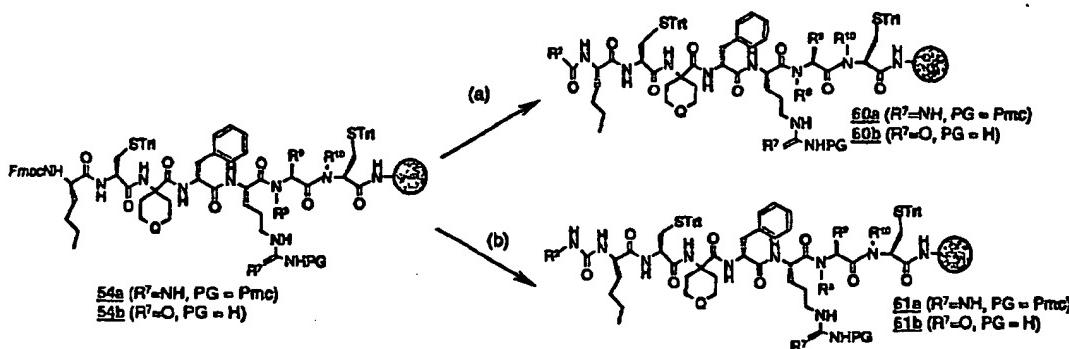
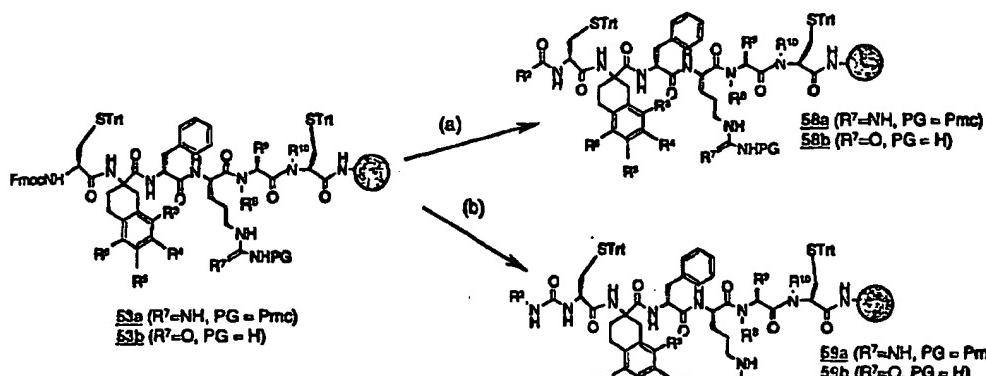
- 41 -

Reaction Scheme L



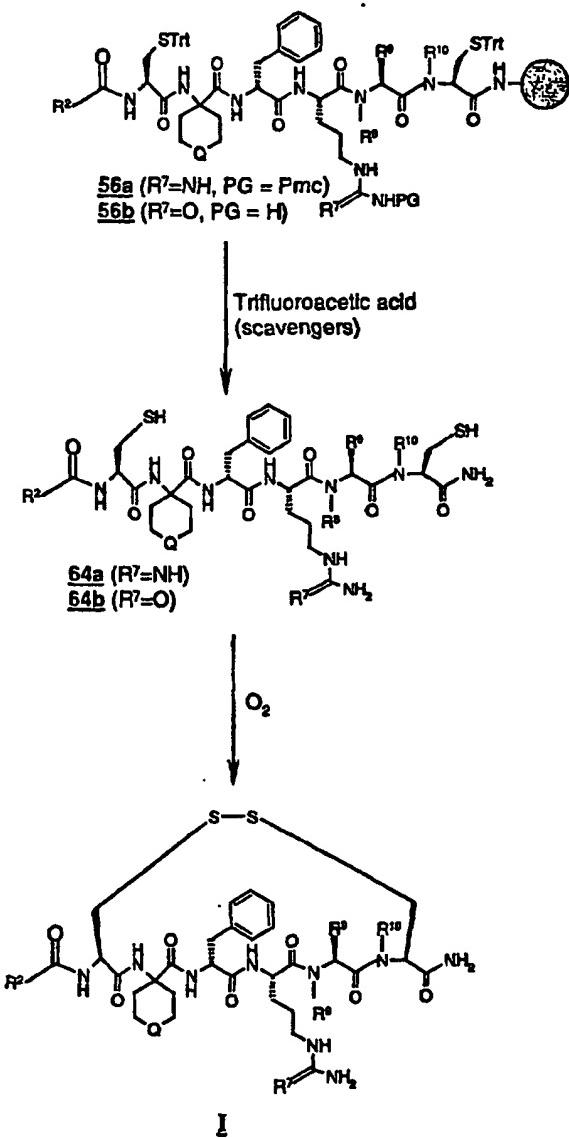
- 42 -

Reaction Scheme M



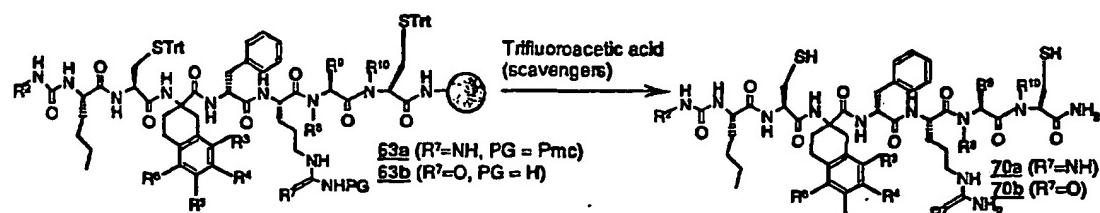
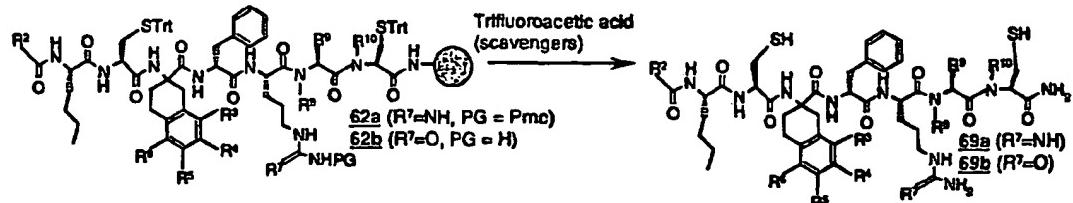
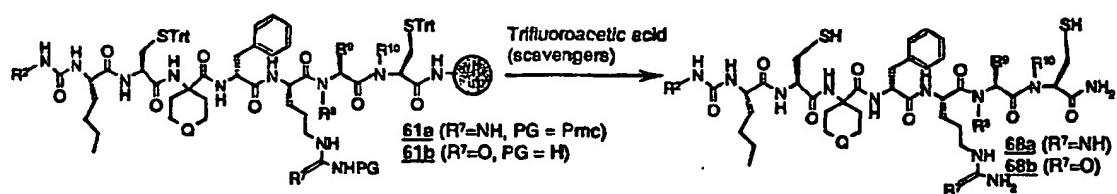
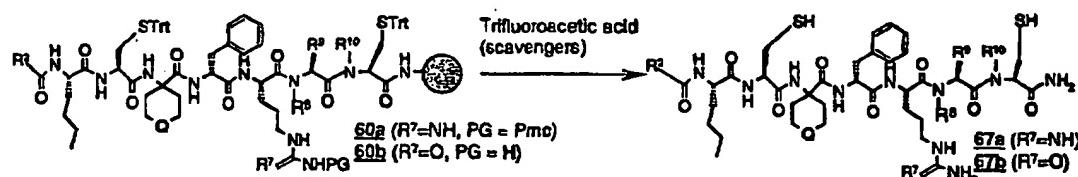
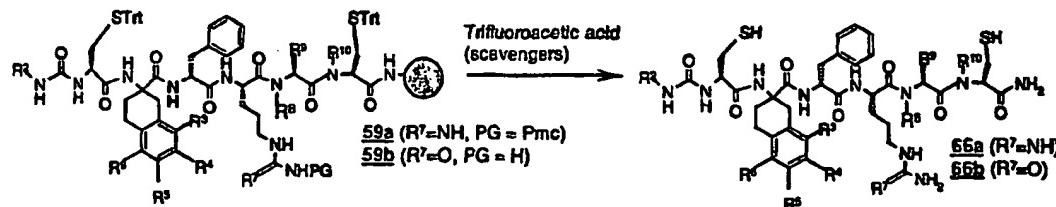
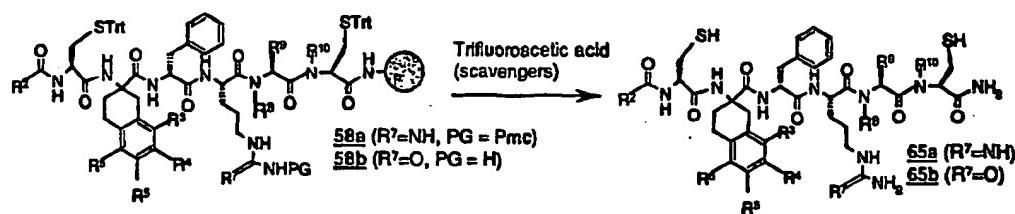
- 43 -

Reaction Scheme N



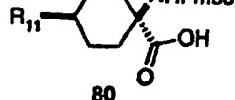
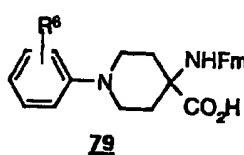
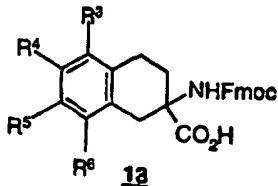
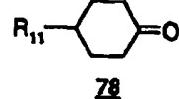
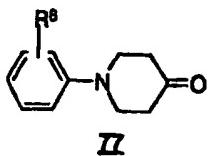
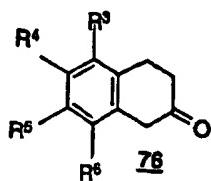
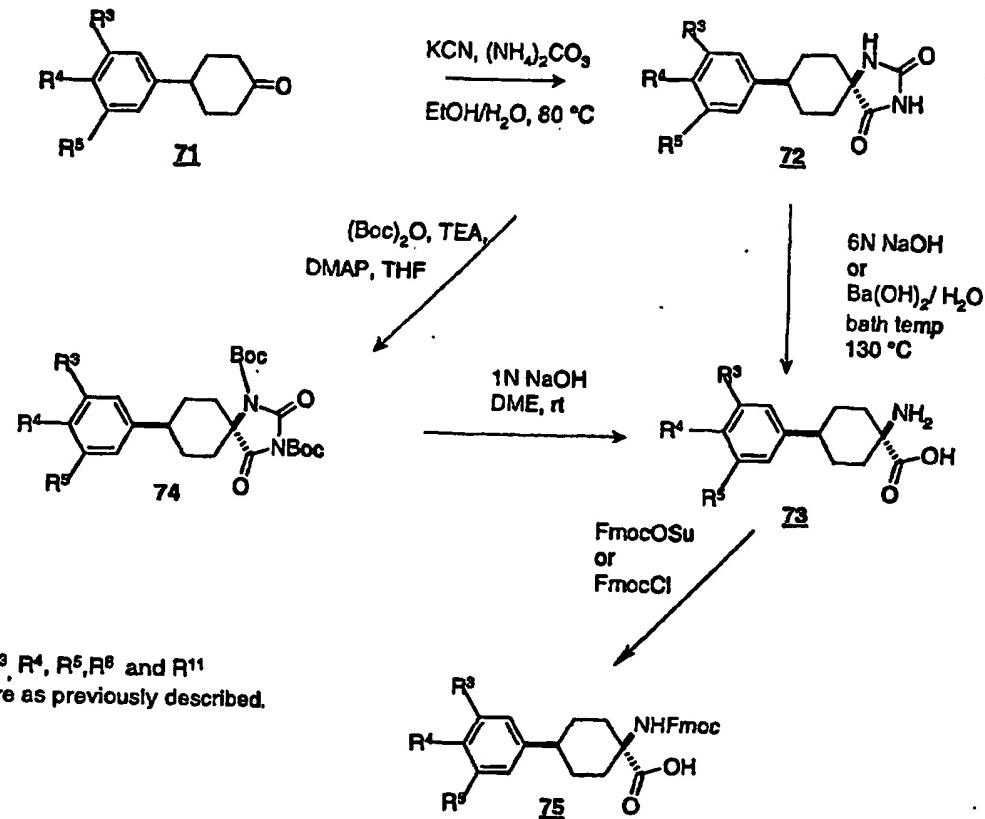
- 44 -

Reaction Scheme O

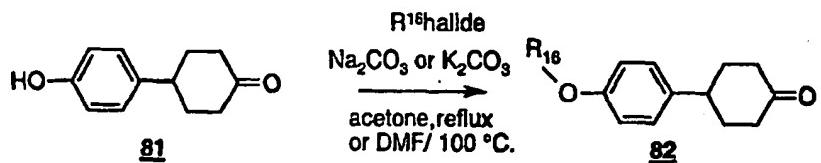


Reaction Scheme Q

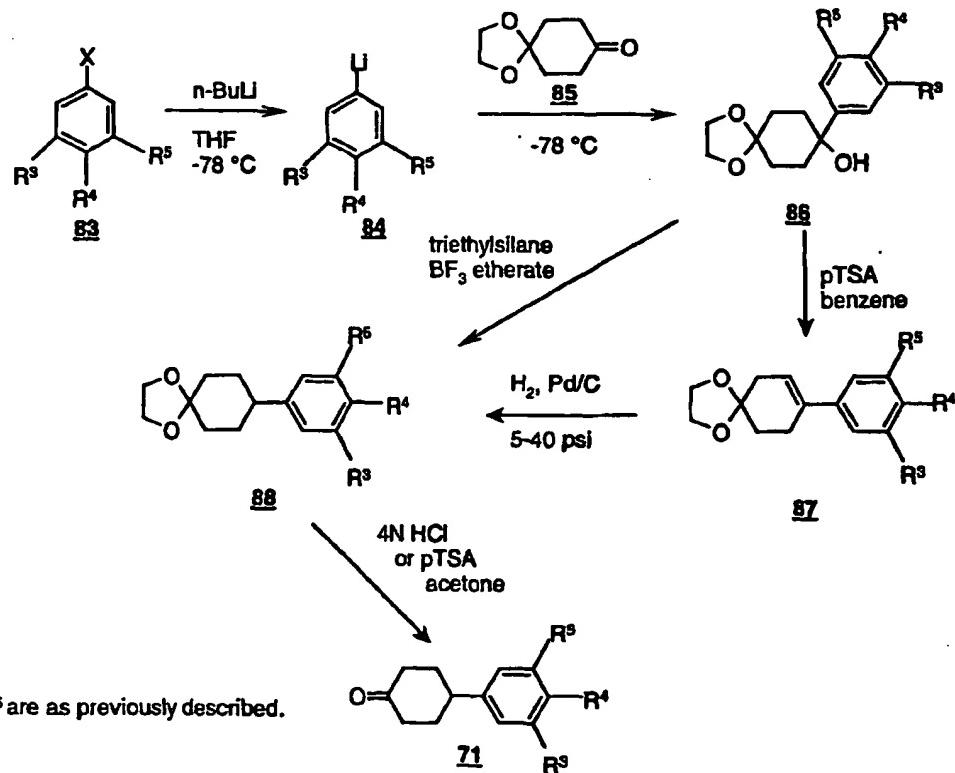
- 45 -



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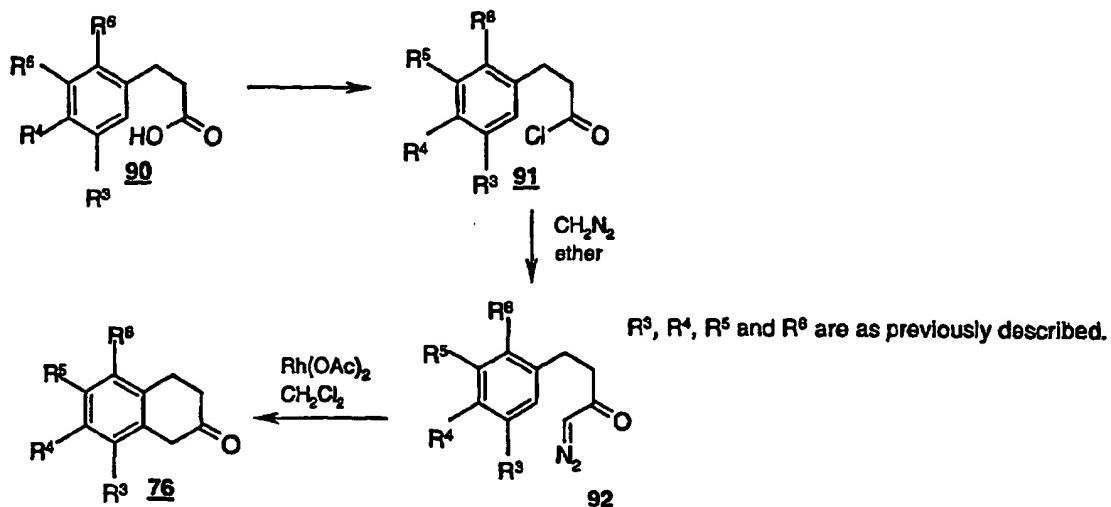
Reaction Scheme R

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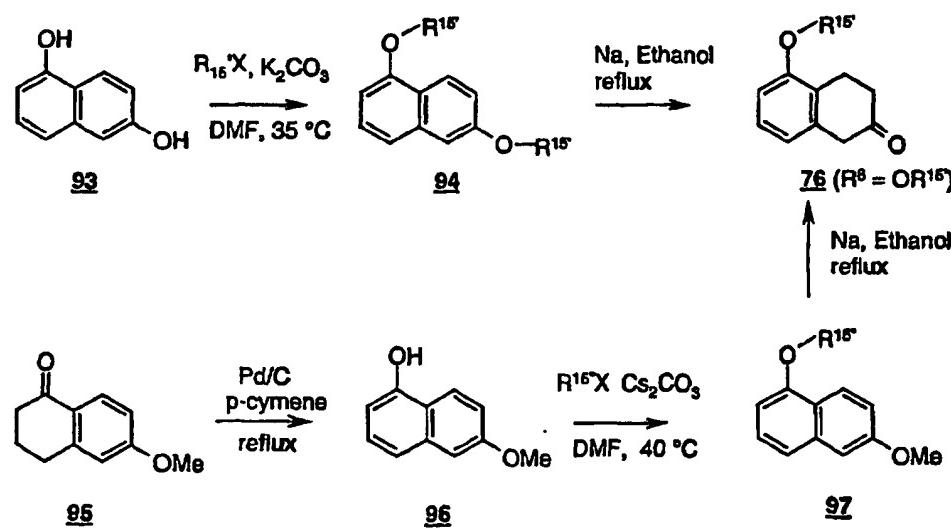
Reaction Scheme S

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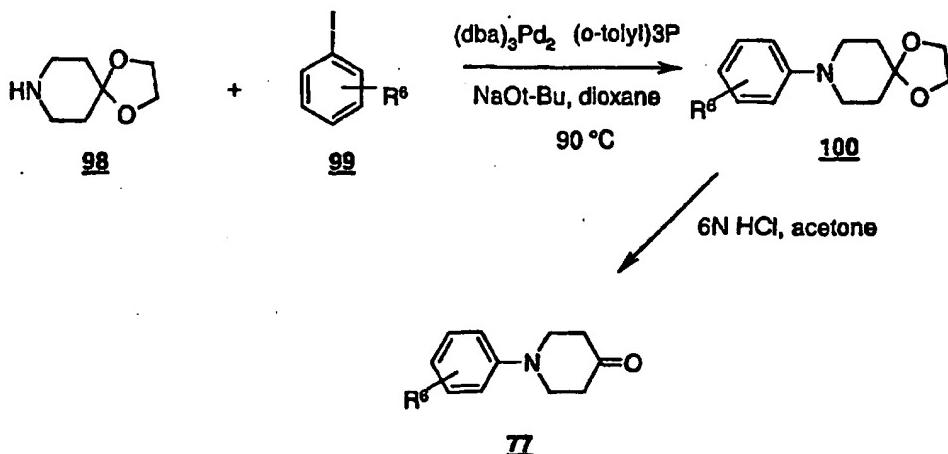
Reaction Scheme T

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Reaction Scheme U

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Reaction Scheme V



R^6 is as previously described.

The linear peptides used herein as the penultimate intermediates in the syntheses of the cyclic peptides of the current invention (Structure 1) are prepared by using conventional solid phase peptide synthesis methodology discussed in the previous section. Each cycle consists of two procedures; the initial cleavage of the Fmoc protecting group from the terminal nitrogen in the resin bound chain followed by acylation of the amine function with an Fmoc protected amino acid. The cycle is generally carried out in accordance with the stepwise procedures outlined in Protocol I. The deprotection is accomplished by using an organic base, for example piperazine, morpholine or piperidine, preferably piperidine in a suitable inert solvent, for example N,N-dimethylformamide (DMF) or N-methylpyrrolidone (NMP). The coupling reaction can be carried out by one of the many conditions developed for amide bond formation, for example O-benzotriazol-1-yl N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) in the presence of an organic base, for example, diisopropylethylamine (DIPEA) in an inert solvent, for example DMF. Alternatively in the present instance, the amide group can be formed using a carbodiimide, for example, diisopropylcarbodiimide (DIC) along with an activating agent such as 1-hydroxybenzotriazole (HOBT) in a suitable inert solvent such as DMF.

In Reaction Scheme A, in the first cycle of the preparation of the linear polypeptides precursors for the cyclic peptides of Structure 1 where Z is NHCO, the Fmoc-Linker-BHA Resin

represented by Structure 2 is deprotected and condensed with Fmoc-amino acids of Structure 2 to give the resin bound compounds of 4. In this syntheses of the cyclic peptides, it is required that the Fmoc-amino acid 3 contain a key structural requirement which is a suitably protected basic side chain, that when deprotected, can participate in the formation of the intramolecular amide bond. To extend the growing peptide chain, a second cycle incorporates the Fmoc-amino acids 5 to give the compounds of Structure 6. In the third cycle, treatment of the resin linked peptide 6 furnishes the intermediates of Structure 7a where R⁸ is hydrogen. The intermediates of Structure 7b where R⁸ is methyl are synthesized as shown in Scheme C.

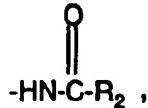
The intermediates of Structure 7b are prepared from the compounds of Structure 7a, as shown in Scheme C. In this procedure, compounds of Structure 7a, prepared by treating compounds of Structure 6 as described in steps 1-5 of Protocol 1, are reacted with an aryl sulfonyl chloride, preferably 2-nitrobenzenesulfonyl chloride 23 to produce the compounds of Structure 24. This reaction is carried out in the presence of a proton acceptor, for example pyridine, triethylamine (TEA) or DIPEA, preferably DIPEA in a suitable inert solvent, preferably DMF. N-methylation of the formed sulfonamide group in the washed resin bound compounds of Structure 24 is accomplished under Mitsunobu conditions to produce the compound of Structure 25. In carrying out this reaction the sulfonamides of Structure 24 are reacted with methanol in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine using methanol as solvent. After the reaction is complete, the resin bound N-methylsulfonamide of Structure 25 is washed free of residual reagents and byproducts.

In the next steps as set forth in Reaction Scheme A the 2-nitrobenzenesulfonyl residue is removed from Structure 25 by reacting 25 with 2-mercaptoethanol and the strong organic base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in a suitable solvent, preferably DMF, to give the resin bound intermediate of Structure 7b. The third cycle of Reaction Scheme A is completed by coupling compounds of either Structures 7a and 7b with Fmoc-Arg(Pmc)-OH (8) or Fmoc-Cit-OH (9) to give the resin bound compounds of Structure 10.

The next two cycles shown in Reaction Scheme B are carried out by first reacting peptides of Structure 10 with the amino acid Fmoc-(D)-Phe-OH to produce the compound of Structure 11 and then reacting this compound of Structure 11 with either one of the amino acid mimetics of Structure 12 or 13 to incorporate these amino acids into the resin bound peptide to give the resin bound pentapeptides of either Structures 14 and 15, depending upon which amino acid mimetics is used. The incorporation of additional amino acid containing carboxylic acid side chains into the linear pentapeptides suitable to ultimately participate in the formation of the cyclic peptides of this invention is accomplished in two ways

- a. As seen in Reaction Scheme B, a Fmoc amino acid with a appropriately protected acid side chain is incorporated into the resin bound pentapeptides 14 and 15. Thus in cycle 6 (Reaction Scheme B) Fmoc-Asp(OtBu)-OH (16) or Fmoc-Glu(OtBu)-OH (17) is incorporated into the growing peptide chain to give the resin bound hexapeptides of Structures 18 and 19 respectively or alternatively,
- 5 b. The resin bound pentapeptides 14 and 15 are N-capped with a cyclic anhydride of Structure 28' (Reaction Scheme F) for example, maleic anhydride or phthalic anhydride, to give the compounds of Structure 29 and 30, or alternatively
- c. As seen in Reaction Scheme B, the resin bound hexapeptides 18 and 19 may be further reacted with an additional amino acid to form a heptapeptide before being N-capped.
- 10 This is accomplished by the incorporation in the usual manner of an amino acid residue, preferably Fmoc-Nle-OH , to furnish 21 and 22.

N-capping of heptapeptide or hexapeptide produces the terminal amide function group of the compound 1. In this manner, the substituents X, Y, R¹² and R¹ are produced. To N-cap the resin bound hexapeptides (18, 19) or heptapeptides (21, 22), the polypeptide is first treated with piperidine in DMF to remove the Fmoc protecting group and then is reacted with an acylating agent. As shown in Reaction Scheme D to produce the compound of Structure 1 before the formation of Z where X and Y are CH, and R¹ is



- 20 the resin bound polypeptide of Structure 18 is deprotected and N-acylated to give the resin bound amide of Structure 27 or after deprotection is reacted with an isocyanate to form the ureas of Structure 28 . The N-acylation is carried out under a variety of methods well known to one skilled in the art. Among the methods used are:
- (i) reaction of the terminal amino functionality with a carboxylic acid R²-CO₂H in a suitable solvent, such as DMF in the presence of HBTU, and an organic base, preferably DIPEA;
- 25 (ii) reaction of the terminal amino functionality with a carboxylic acid chloride R₂-COCl in a suitable solvent, such as dichloromethane in the presence of an organic base, such as pyridine, TEA and DIPEA, preferably DIPEA; or
- (iii) reaction of the terminal amino functionality with a carboxylic acid anhydride of Structure

- 51 -

28' as shown in Reaction Scheme F. This Reaction is carried out in a suitable solvent, such as dichloromethane or DMF in the presence of an organic base, preferably DIPEA.

The N-capping reaction in Reaction Scheme D where compounds of Structure 18 are converted to ureas of Structure 28 is carried out by reacting the terminal amino group in the 5 compounds of Structure 28 with an isocyanate R²-NCO. This reaction is carried out in a suitable solvent, such as dichloromethane or DMF in the presence an organic base, preferably DIPEA. When the acylation and urea forming reactions are complete, the resin bound products 27 and 28 are washed free of residual reagents and byproducts. By using similar conditions, N-capping of the resin bound polypeptides of Structure 19, 21, and 22 is carried out by fomation of the N- 10 acylated compounds of Structures 33, 35, 37 and the ureas of Structure 34, 36 and 38 (Reaction Scheme E). However, Reaction Scheme E can be modified to provide R¹⁴ groups other than that derived from Nle by utilizing known amino acids other than the compound of Structure 20 in Reaction Scheme B to produce compounds of Structures 21 and 22.

Reaction Schemes G and H illustrate the cleavage of remaining protecting groups in the N- 15 capped polypeptides 29, 30, 33 - 38 and the concomitant cleavage of the peptides from the solid support. This reaction is carried out by using a strong organic acid, preferably trifluoroacetic acid, optionally in the presence of an inert solvent such as dichloromethane and a trace (1%) of water and optionally in the presence of one or more carbocation scavengers, for example ethanedithiol, dimethyl sulfide, triethylsilane and anisole. The polypeptide cleavage solution is 20 filtered free from the solid support, then is diluted with a suitable solvent, preferably diethyl ether, and the formed solids are collected by filtration. The solid polypeptides of Structures 39-44 produced in Reaction Scheme H may be purified by reversed phase chromatography by using a preparative C18 column.

With the appropriate functionalities thus now available to form the intramolecular amide bond, the N-capped linear polypeptides are subjected to amide forming reaction conditions well 25 known in the art. Accordingly each in turn of the the linear peptides 31, 32 (Reaction Scheme G) and 39-44 (Reaction Scheme I) is dissolved in an inert solvent, for example DMSO, and by the addition of a tertiary amine base, for example N-methyl morpholine the solution is adjusted to an apparent pH of 8, prior to the addition of an amide forming reagent, for example BOP. The 30 reaction is conveniently carried out at a temperature of between zero degrees and 40 °C, preferably at about room temperature. The reaction is allowed to proceed until it is judged to be complete. Common methods, used by those of average skill, to monitor the progress of a reaction are for example, TLC or analytical HPLC. After removal of the reaction solvents *in vacuo* the crude cyclic peptides of Structure I where Z is NHCO as shown in Reaction Scheme I may be

purified by reversed phase chromatography using a preparative C18 column. In this manner Compound of Structure 1 are produced where Z is NHCO Bridge.

- The cyclic disulfide peptides of Structure 1 where Z is S-S are prepared by methodologies, outlined in Reaction Schemes similar to those described above in Reaction Schemes A through H
- 5 for the preparation of the lactams of Structure 1 where Z is NHCO. The penultimate linear polypeptides are assembled in a like manner, with the exception that amino acids, that contain protected thiol side chain residues, for example Fmoc-Cys(Trt)-OH, are incorporated at the appropriate positions of the growing resin bound polypeptide, preferably in cycle 1 and 6. This preparation of the resin bound linear polypeptides is illustrated in Reaction Schemes J and K. As
- 10 previously described and shown in Scheme L, the resin bound linear hexapeptide 52, after removal of the Fmoc protecting group group, may be N-capped either by acylation to give 56 or by reaction with an isocyanate to form the urea 57. In the same way the resin bound hexapeptide 53, and the resin bound heptapeptides 54 and 55 are converted to the corresponding N-acylated derivatives 58, 60 and 62 and the urea derivatives 59, 61 and 63 (Reaction Scheme M).
- 15 In Reaction Scheme N, the N-capped resin bound linear hexapeptide 56 is treated with a strong acid, preferably trifluoroacetic acid, optionally in the presence of an inert solvent such as dichloromethane and optionally in the presence of one or more carbocation scavengers, for example ethanedithiol, dimethyl sulfide, triethylsilane and anisole. This causes cleavage of all side chain protecting groups as well as cleavage of the linear peptide from the solid support. The
- 20 reaction is conveniently carried out at a temperature of between 0 °C and 35 °C, preferably at room temperature. The polypeptide cleavage solution is filtered free from the solid support, then is diluted with a suitable solvent, preferably diethyl ether, and the formed solids are collected by filtration. The solid polypeptide of Structure 64 thus produced optionally may be purified by reversed phase chromatography by using a preparative C18 column. The linear
- 25 hexapeptide 64 is then treated under oxidative conditions well known to one skilled in the art, that can induce thiols to form a disulfide bond. Accordingly a dilute aqueous solution of 64 is adjusted to pH 8.0 using a moderately weak inorganic base, preferably ammonium hydroxide, and then oxygen was bubbled into the solution until the cyclization was judged to be complete, using standard methods, for example TLC or HPLC. After removal of the reaction solvents by
- 30 lyophilization, the crude cyclic peptide (I; Z= S-S) prepared and isolated in this manner, may be purified by reversed phase chromatography by using a preparative C18 column.

Under similar conditions to those described hereinbefore in connection with Reaction Schemes H and I, the resin bound linear polypeptides 58-63 as shown in Reaction Schemes O and P, are deprotected and cleaved from the solid support to give the linear peptides 65-70

(Reaction Scheme O), which are then oxidatively cyclized, as described above, to give the corresponding compounds of Structure 1 (Reaction Scheme P).

from zero degrees to room temperature, preferably at room temperature. In the same manner, as in Reaction scheme Q, the tetralones 76, the N-aryl-4-ketopiperidines 77, and the cyclohexanone derivatives 78 can be converted to the corresponding Fmoc-amino acids of Structures 13, 79 and 80, all of which along with 73 form subgenera of Structure 12 utilized in Reaction Scheme B.

- 5 In Reaction Scheme Q, the compounds of Structure 73 where R⁴ represents a linear or branched lower alkoxy and both R² and R³ is hydrogen in the sub genus Structure 82, may be prepared by O-alkylation of the compound of Structure 81 as shown in Reaction Scheme R. Where R¹⁶ represents an unbranched lower alkyl moiety, the alkylation is carried out by using a primary alkyl halide of Structure R¹⁶halide in the presence of an alkali metal carbonate, for example, sodium or potassium carbonate. The alkyl halide may be a chloro, bromo or iodo derivative, preferably an alkyl iodide. The reaction may be conveniently carried out in an inert solvent that promotes SN₂ displacement reactions, for example acetone, 2-butanone or N,N-dimethylformamide, preferably acetone, at a temperature of from room temperature to the reflux temperature of the solution, preferably the reflux temperature. When R¹⁶ represents a branched lower alkyl group, e.g., 2-propyl, the alkylation of the compound of Structure 81 to produce the compound of Structure 82 is carried out by using a secondary alkyl halide of Structure R¹⁶halide in the presence of an alkali metal carbonate, e.g., potassium carbonate. The secondary alkyl halide is preferably a secondary alkyl iodide, for example, 2-iodopropane. The reaction may be conveniently carried out in an inert solvent, preferably N,N-dimethylformamide, at a temperature of from room temperature to the reflux temperature of the solution, preferably at about 100 °C.

- 20 4-Arylcyclohexanones of Structure 71 which are the starting materials in Reaction Scheme Q can be prepared by methods that are well known to one of ordinary skill in the practice of organic chemistry. As outlined in Scheme S, treatment of the aryl halides of Structure 83, where X¹ represents bromo or iodo with an alkyl metal reagent, preferably t-butyl lithium, results in a transmetalation reaction to give the corresponding aryl lithium of Structure 84. The reaction is conveniently carried out at -78 °C by the addition of a solution of the alkyl lithium into a solution of a compound of Structure 83 in an inert anhydrous solvent, such as diethyl ether or tetrahydrofuran, preferably tetrahydrofuran. The aryl lithium of Structure 84 produced in this manner, is then reacted *in situ* with a solution of the monoketal of cyclohexane-1,4-dione (85) in an suitable inert solvent, for example tetrahydrofuran, while the reaction temperature is maintained below -60 °C, preferably at about -78 °C to give the carbinols of Structure 86. The compounds of Structure 87 are obtained by the dehydration of the carbinols of Structure 86. The reaction is conveniently carried out using a strong organic acid catalyst, preferably p-toluenesulfonic acid in an inert solvent, for example benzene or toluene, preferably benzene, at

- the reflux temperature of the solvent. The formed water is removed from the reaction mixture by means of a Dean Stark apparatus to enable the reaction to go to completion. Compounds of Structure 88 are produced by hydrogenation of the olefins of Structure 87. The reaction is conveniently carried out using a noble metal catalyst, for example palladium on carbon, in a 5 hydrogen atmosphere in an inert solvent, for example ethanol or ethyl acetate. The hydrogenation is usually carried out at room temperature and 40 psi of hydrogen, however if the aryl ring in Structure 87 contains a group prone to hydrogenolysis, e.g., if R³, R⁴ or R⁵ represents chloro, the reaction pressure is kept at about 5 psi. Compounds of Structure 88 may be also obtained directly from carbinols of Structure 86 by reductive elimination of the hydroxyl group.
- 10 In this reaction a solution of the compound of Structure 86 (R³=R⁴=H and R⁵=OMe) in an inert solvent, for example dichloromethane, is treated with a Lewis acid, such as boron trifluoride etherate, and a reducing agent, for example triethylsilane, at a temperature of from zero degrees to room temperature. Removal of the ketal protecting group in compounds of Structure 88 gives the ketone of formula 71, which is the starting material for Reaction Scheme Q
- 15 for preparing the Fmoc-amino acid species of Structure 75 of the compound of Structure 12. The reaction is conveniently carried out in acetone or 2-butanone, preferably acetone under acid catalysis, for example 4N hydrochloric acid or p-toluenesulfonic acid at from room temperature to the reflux temperature of the reaction mixture, preferably at the reflux temperature.

- 5-Substituted-beta-tetralones of Structure 76 shown in Reaction Scheme Q which is the 20 starting material for preparing compound of Struture 13 are known compounds, or if they are not known they can be prepared by methods that are well known to one of ordinary skill in the field of organic chemistry. In the present instance, compounds of Structure 76 are basically prepared by two methods outlined in Reaction Schemes T and U. As shown in Scheme T, a 2- substituted hydrocinnamic acid of Structure 90 is converted to the corresponding carboxylic acid 25 chloride of Structure 91. This conversion can be carried out by several methods, for example by treatment of the hydrocinnamic acid with oxalyl chloride, optionally in the presence of a catalytic amount of N,N-dimethylformamide, in an inert solvent, such as benzene or dichloromethane, preferably dichloromethane. The reaction may be conveniently carried out at a temperature of from zero degrees to room temperature, preferably at room temperature. Alternatively the 30 compound of Structure 90 is reacted with an acyl chloride forming reagent such as sulfonyl chloride in an inert solvent, for example benzene or toluene, preferably toluene at a temperature between room temperature to the reflux temperature of the solution, preferably at the reflux temperature. The diazoketone of Structure 92 is prepared by treatment of the thus formed acyl halide of Structure 91 in an inert solvent, e.g., dichloromethane with an excess of a freshly 35 prepared ethereal solution of diazomethane. The combination of reagents is conveniently carried out at ice bath temperature and the reaction is then allowed to proceed at a temperature of from

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zero degrees to room temperature, preferably at room temperature. As shown in Reaction Scheme T, cyclization of the diazoketone of Structure 92 to furnish the tetralone of Structure 76 is promoted by rhodium (II) acetate dimer in an inert solvent, e.g., dichloromethane. The reaction is normally carried out at from room temperature to the reflux temperature of the
5 solution, preferably at the reflux temperature.

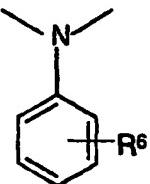
Compounds of Structure 76, which is a starting material in Reaction Scheme Q, wherein R⁶ represents a linear or branched lower alkoxy group can be prepared as shown in Reaction Scheme U from compounds of Structure 93. In Reaction Scheme U, the compounds of Structure 94 where R¹⁵⁰ is an unbranched lower alkyl moiety are prepared by per-O-alkylation of the
10 naphthalenediol of Structure 93 with a primary alkyl iodide or bromide, preferably an iodide, in the presence of a base such as an alkali metal carbonate, for example, sodium or potassium carbonate. The reaction may be carried out in an inert solvent, preferably N,N-dimethylformamide at a temperature of from room temperature to 100 °C, preferably at 35 °C. The compounds of Structure 97 where R¹⁵⁰ is a branched lower alkyl are prepared in two steps
15 from the 2-tetralone of Structure 94. The tetralone of Structure 95 is subjected to dehydrogenation in the presence of a noble metal catalyst, such as palladium metal (10% on carbon) in a suitable high boiling solvent such as p-cymene to give the aromatized compound of Structure 96. The naphthol of Structure 96 is then O-alkylated with a secondary alkyl iodide in the presence of a base such as an alkali metal carbonate, preferably cesium carbonate to furnish
20 the compound of Structure 97. The reaction may be conveniently carried out in an inert solvent, preferably N,N-dimethylformamide at a temperature of from room temperature to 100 °C, preferably at about 40 °C.

The tetralones of Structures 76 are produced by reduction of the compounds of Structures 94 and 97 under dissolving metal conditions, followed by the acid catalyzed hydrolysis
25 of the intermediate enol ethers. The transformation is conveniently carried out by the portionwise addition of a large excess of an alkali metal, such as sodium or potassium, preferably sodium, to a boiling solution of the substrate in a lower alcohol, preferably ethanol until the starting material is consumed. The tetralones of Structures 76 are obtained by treatment of a solution of the isolated intermediate enol ethers with a strong acid catalyst, preferably p-toluenesulfonic acid. The hydrolysis may be conveniently carried out in a mixture of a lower
30 alcohol, preferably ethanol, and water at a temperature of between room temperature and the reflux temperature of the solution, preferably at the reflux temperature.

In preparing the compounds of Structure 77, utilized in Reaction Scheme Q as starting materials in producing the compounds of formula 79 which are the Fmoc-amino acid species of

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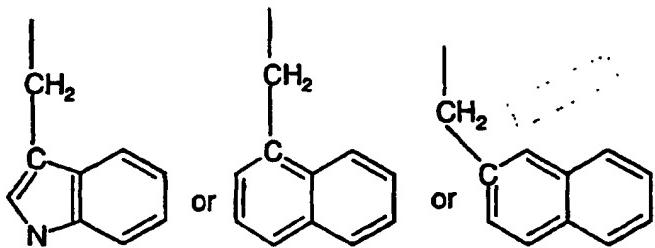
the compound of Structure 12, the compound of Structure 98 is used as a starting material. This is shown in Reaction Scheme V. In Reaction Scheme V, compounds of Structure 100 can be prepared by reactions that are known *per se*. For example, they can be prepared by coupling the secondary amine of Structure 98 with an aryl bromide or iodide, preferably an aryl iodide of Structure 99 (Reaction Scheme V). The coupling reaction is catalyzed by a noble metal catalyst, preferably tri(dibenzylideneacetone)dipalladium, in the presence of a chelating phosphine ligand, preferably tri-*o*-tolylphosphine, and a hindered alkoxide base such as sodium tert-butoxide. The reaction is conveniently carried out in an inert atmosphere using an anhydrous solvent such as dioxane or toluene, preferably dioxane, at a temperature of from 60 °C to the reflux temperature, preferably at 90 °C. Removal of the carbonyl protecting group in compound 100 to give compounds of Structure 77 can be carried out by a variety of methods well known in the field of organic chemistry. For example, the deprotection can be achieved by treatment of a solution of compound 100 in a low boiling ketone such as acetone or 2-butanone with an aqueous mineral acid solution, for example 6N hydrochloric acid at a temperature of from room temperature to the reflux temperature of the mixture, preferably at the reflux temperature. The compound of Structure 100 when treated in this manner with an aqueous mineral acid forms, in Reaction Scheme V, the compound of Structure 77. The compound of Structure 77, as demonstrated in the discussion of Reaction Scheme Q is the intermediate for the compound of Structure 79 which is the species of acid of Structure 12 wherein Q is



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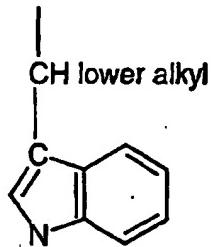
In Reaction Scheme A, amino acids of Structure 5 are known compounds where

R⁹ is



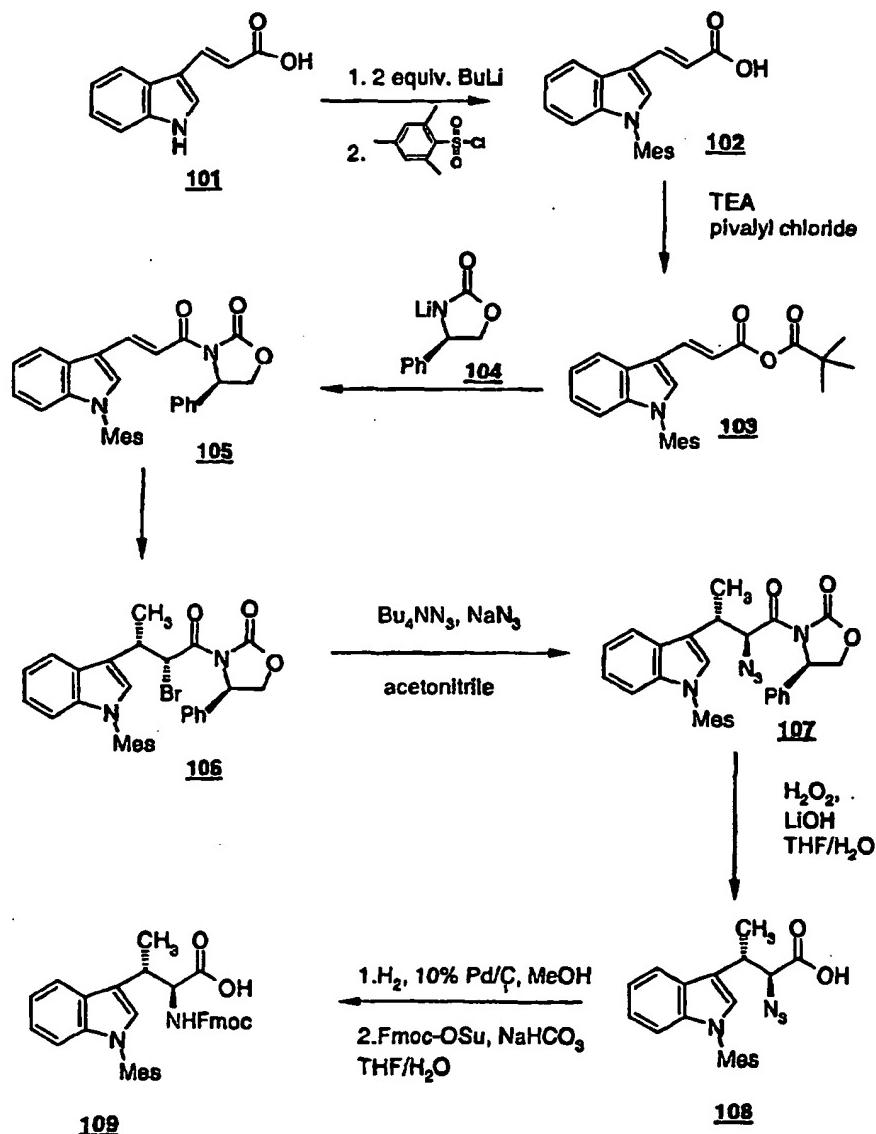
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On the other hand, amino acids of Structure **5** where R¹⁷ in R⁹ is lower alkyl, i.e., where R⁹ is



are prepared from a compound of formula **101** via Reaction Scheme V as follows:

Reaction Scheme W



As shown in Reaction Scheme W, β -methyl(Nin-Mes)tryptophan 109 was prepared by using the method previously described by Boteju, L.W., Wenger K., and Hruby, V.J. *Tet.Lett.*, **33**, 7491 (1992). In the first step the nitrogen in *trans*-indole-3-acrylic acid 101 is protected by conversion to the corresponding mesitylenesulfonamide 102. The reaction is carried out by treating the 5 indole acid 101 with a excess (> 2 equiv.) of a solution of an alkyl or aryl lithium reagent, for example phenyl lithium or n-butyllithium, preferably n-butyllithium in an inert solvent, preferably tetrahydrofuran, at a temperature of from -40 °C to about -100 °C, most conveniently at -78 °C. While the reaction is maintained at about -78 °C, the formed dilithiated species is then reacted with mesitylenesulfonyl chloride to furnish the mesitylenesulfonamide 102. The N- 10 protected indole acrylic acid 102 is then coupled with the chiral auxiliary (R)-4-phenyl-2-oxazolidinone (preparation see Nicolas et al., *J. Org Chem.* 1993, **58**, 766-770.) as its N-lithiated species 104 to give the chiral acrylamide 105. The coupling was accomplished via a mixed anhydride formed from 102. To form the mixed anhydride 103 the N-protected indole acrylic acid 102 was reacted with a suitable acyl chloride, for example t-butyl chloroformate, 2,4,6 15 trichlorobenzoyl chloride or pivaloyl chloride, preferably pivaloyl chloride in the presence of an tertiary amine base, for example triethylamine or diisopropylethylamine, preferably triethylamine. The criteria that make possible the choice of a suitable acyl chloride to form the anhydride 103 are well established and are known by one of ordinary skill in organic chemistry. The anhydride formation is run in an inert solvent, for example tetrahydrofuran at an initial 20 temperature of between -100 °C and 0 °C, preferably at about -78 °C. The reaction is allowed to go to completion at a temperature of between -78 °C and 0 °C degrees, preferably at about 0 °C. The thus formed mixed anhydride 103 is then reacted *in situ* with a solution of N-lithiated (R)-4-phenyl-2-oxazolidinone 104, previously prepared by treating a solution of (R)-4-phenyl-2-oxazolidinone in an inert solvent, for example tetrahydrofuran, with an equimolar amount of a 25 solution of an alkyl or aryl lithium reagent, for example phenyl lithium or n-butyllithium, preferably n-butyllithium in an inert solvent, for example, hexane at a temperature of between -100 °C and zero degrees, preferably at about -78 °C. The coupling reaction which yields the chiral acrylamide 105 is carried out at an initial temperature of between -100 °C and zero degrees, preferably at about -78 °C, and after all the reagents are combined the reaction is 30 allowed to proceed at a temperature of between -78 °C and room temperature, preferably at about room temperature.

The highlight of the transformation of the chiral acrylamide 105 into 106, is the controlled generation of the two new contiguous chiral centers present in 106. The reaction involves the stereoselective 1,4-conjugate addition (Michael addition) of a methyl cuprate, 35 formed *in situ* from cuprous bromide-dimethylsulfide complex and methyl magnesium bromide,

to the Micheal acceptor, the α,β -unsaturated carbonyl system present in 105. The resultant metal-chelated enolate is then directly halogenated with a halogenating agent, preferably N-bromosuccinimide, to give 106. Again, as in the case of the Michael addition, the incorporation of the bromo atom is stereoselective, controlled by the bulky phenyl group on the chiral auxiliary

5 which effectively shields the *si* face of both the α,β -unsaturated acyloxazolidinone system and the intermediate metal-chelated enolate from attack from incoming reagents. To prepare the methyl cuprate, a solution of methyl magnesium bromide in diethyl ether is added to a solution of cuprous bromide-dimethylsulfide (1:1) complex in an inert solvent, for example dimethyl sulfide or tetrahydrofuran, preferably a mixture thereof. The reaction is run at a temperature of

10 between -78°C and room temperature, preferably at -4°C . Instead of methyl magnesium bromide, any lower alkyl bromide can be added to form R^{17} where R^{17} is a lower alkyl group other than methyl. To this formed solution of methyl cuprate, is added *in situ* a solution of the α,β -unsaturated acyloxazolidinone 105 in an inert solvent, preferably tetrahydrofuran. The methyl cuprate addition is run initially at a temperature of between -30°C and room

15 temperature, preferably at -4°C and then is allowed to proceed at room temperature. When it is judged that the reaction is completed (e.g. analysis by TLC or HPLC), it is then cooled to a temperature of between -100°C and -40°C , preferably at about -78°C , whereupon a solution of a halogenating agent, preferably N-bromosuccinimide in an inert solvent, for example, tetrahydrofuran is added. The reaction is then allowed to proceed at a temperature of between 0

20 $^{\circ}\text{C}$ and room temperature, preferably at about room temperature to give after isolation, the bromide 106. The bromide is displaced by azide ion with a concomitant inversion of configuration. This transformation is accomplished by reaction of the bromide 106 with tetrabutylammonium azide in the presence of excess sodium azide in an inert solvent, for example acetonitrile to give the azide 107. The reaction is conveniently run at a temperature of between

25 80°C and room temperature, preferably at about room temperature. Treatment of 107 and an alkali metal hydroxide, or example sodium, potassium or lithium hydroxide, preferably lithium hydroxide in the presence of hydrogen peroxide resulted in the hydrolysis of the chiral auxilliary to give the azido acid 108. The hydrolysis reaction is run in an inert solvent, preferably water, at a temperature of between 0°C and room temperature, preferably at about 0°C . Hydrogenation of

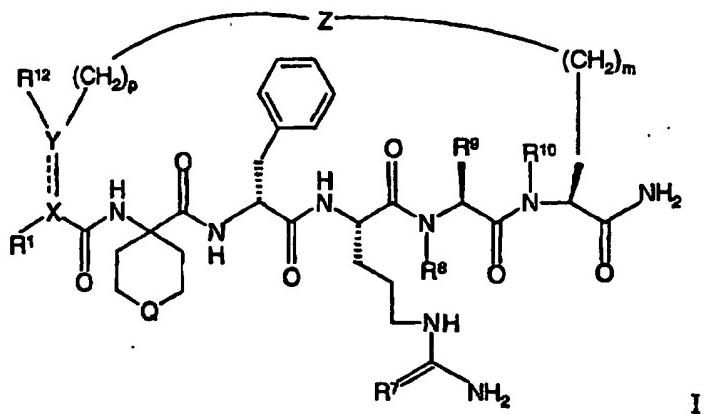
30 the α -azido acid 108 gives the β -methyl(Nin-Mes)tryptophan which is immediately converted to the corresponding N(α)-Fmoc- β -methyl(Nin-Mes)tryptophan 109. The hydrogenation of 108 is carried out over a noble metal catalyst, preferably 10% Pd/C, in an inert solvent, for example a lower alkanol, preferably methanol at low pressure (< 2 atmos) and at room temperature. After removal of the catalyst by filtration, the volatiles are removed and the product is dissolved in an

35 inert solvent, for example tetrahydrofuran or water, preferably a mixture thereof, and treated with a mild inorganic base, for example an alkali metal bicarbonate, preferably sodium

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bicarbonate, and a Fmoc N-protecting group forming reagent, for example 9-fluorenylmethyl chloroformate (Fmoc-Cl) or 9-fluorenylmethyl-N-succinimidyl carbonate (Fmoc-OSu), preferably Fmoc-OSu to furnish 109. The reaction is conveniently run at a temperature of between 0 °C and room temperature, preferably at about room temperature. The compound of formula 109 is a species of amino acid of Structure 5 in Reaction Scheme A.

The present invention refers also to a process for the preparation of a compound of the formula:



wherein R¹ to R¹², m, p, Q, X, Y and Z are as defined above by formation of a lactam bond or a disulfide bond at the Z position of the precursor linear peptides.

Pharmaceutical Compositions

The present invention also refers to pharmaceutical compositions comprising compounds as defined above and a therapeutical inert carrier.

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The compounds of formulas I and II as well as Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Ala-Trp-Lys-NH₂; and Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Arg-(2S,3S) beta methyl-Trp-Lys-NH₂ prepared in accordance with this invention may be prepared in pharmaceutical compositions suitable for administration or inhalation with a suitable carrier or vehicle by methods known in the art.

The compounds as described above can be used as medicaments, e.g. in the form of pharmaceutical preparations, e.g. for parenteral administration. They can be administered, for example, parenterally, e.g. in the form of injection solutions or infusion solutions.

The production of the pharmaceutical preparations can be effected in a manner which will

be familiar to any person skilled in the art by bringing the compounds as described above, optionally in combination with other therapeutically valuable substances, into an administration form together with suitable, non-toxic, inert, therapeutically compatible solid or liquid carrier materials and, if desired, usual pharmaceutical adjuvants.

- 5 Suitable carrier materials are not only inorganic carrier materials, but also organic carrier materials.

- Usual stabilizers, preservatives, wetting and emulsifying agents, consistency-improving agents, flavour-improving agents, salts for varying the osmotic pressure, buffer substances, solubilizers, colorants and masking agents and antioxidants come into consideration as
10 pharmaceutical adjuvants.

- The dosage of the compounds as described above can vary within wide limits depending on the disease to be controlled, the age and the individual condition of the patient and the mode of administration, and will, of course, be fitted to the individual requirements in each particular case. For adult patients a daily dosage of about 1 mg to about 1000 mg, especially about 10 mg to
15 about 500 mg, comes into consideration. Depending on the dosage it is convenient to administer the daily dosage in several dosage units.

The pharmaceutical preparations conveniently contain about 1-500 mg, preferably 5-200 mg, of a compound as described above.

20 Treating Obesity

- The compounds prepared in accordance with this invention exhibited selective MC-4 receptor agonist activity *in vitro*. It is known that agonists of MC4-R activity cause reduction of food intake in a mouse model of human obesity. Therefore administration of these compounds agonizes MC4-R activity which is important in the regulation of body weight. The
25 pharmaceutical compositions containing the compounds of this invention may be formulated at a strength effective for administration by various means to a human or animal patient experiencing undesirably elevated body weight, either alone or as part of an adverse medical condition or disease, such as type II diabetes mellitus. A variety of administrative techniques can be used. Average quantities of the active compound may vary and in particular should be based
30 upon the recommendations and prescription of a qualified physician or veterinarian.

Accordingly, the present invention also relates to the use of compounds as defined above for the preparation of medicaments for the treatment and/or prophylaxis of diseases which are

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- associated with melanocortin-4-receptor activity. Further, the invention relates to a method for the treatment and/or prophylaxis of diseases which are associated with melanocortin-4-receptor activity, which method comprises administering a compound as defined above to a human being or animal. The compounds are especially useful for the treatment and/or prophylaxis of obesity.
- 5 The invention also refers to a compound as defined above when manufactured by a process as described above. In addition the invention refers to compounds as defined above for use as therapeutic active substances, particularly as therapeutic active substances for the treatment and/or prophylaxis of diseases which are associated with melanocortin-4-receptor, e.g. obesity.

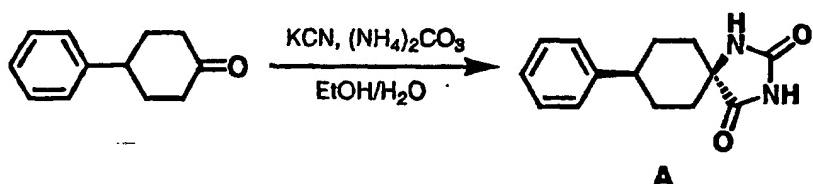
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This invention will be better understood by reference to the following examples, which illustrate but do not limit the invention described herein. In the structures of specific compounds appearing in the Examples section, the hydrogens have generally been omitted for convenience.

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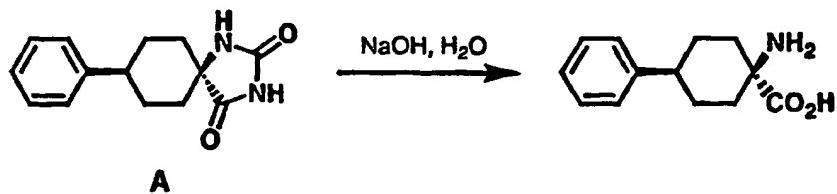
EXAMPLESEXAMPLE 1Preparation of Fmoc-1-amino-4-phenylcyclohexane-1-carboxylic acid (Fmoc-Apc)**Step 1:**

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To a solution of 4-phenylcyclohexanone (10.0 g, 57.5 mmol) in ethanol (100 mL) and water (33 mL) in a glass pressure bottle, were added ammonium carbonate (33 g, 344 mmol, 6 equiv.) and potassium cyanide (5.6 g, 86.2 mmol, 1.5 equiv.). The mixture was heated at 80-90
10 °C for 24 hrs. The cooled reaction mixture was added to icy water (400 ml) and stirred vigorously for 30 min. The resulting precipitate was suction filtered, washed thoroughly with water and dried to yield the hydantoin A as a white solid (14.0 g, 100% yield). ¹H NMR (DMSO-d₆): 8.63 (s, 1H), 7.23-7.36 (m, 4), 7.15 (m, 1), 2.50 (m, 1H), 2.10 (m, 1H), 1.85 (d, 1H) and 1.55-1.80 (m, 6H).

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Step 2:

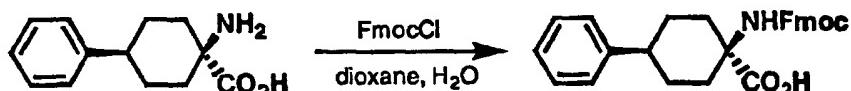
The hydantoin A (10.0 g) was suspended in aqueous NaOH (6N, 350 mL) and heated at
20 130 °C for 2-3 days. Upon the completion of the hydrolysis, the reaction mixture was neutralized with conc. HCl to slightly acidic (pH ~6). The resulting slurry was filtered, washed with water and dried to give 1-amino-4-phenylcyclohexane carboxylic acid (APC) as a white

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solid (25 g, >100 % yield, contaminated with inorganic salt) which was used directly for next step. Small portion of the crude product was purified on HPLC. ^1H NMR (DMSO-d₆): 7.23~7.735 (m, 2), 7.10~7.19 (m, 3H), 2.45 (m, 1H), 1.92~2.18 (m, 3H), 1.56~1.78 (m, 4H) and 1.20 (m, 1H); LRMS (electrospray) *m/e* 220 (M+1)⁺, Calcd for C₁₃H₁₇NO₂, 219.

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Step 3:



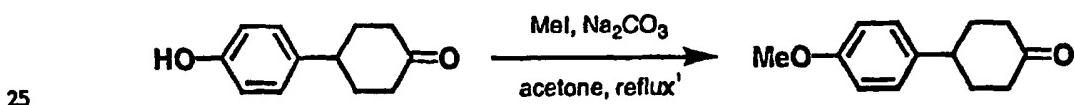
The crude 1-amino-4-phenylcyclohexane carboxylic acid (APC) from the last step (25 g) was suspended in dioxane (300 mL) and aqueous 10 % Na₂CO₃ (150 ml) and stirred vigorously 10 overnight. The reaction mixture was concentrated to remove dioxane, neutralized with 6N HCl to slightly acidic (pH 5-6) and extracted with EtOAc. The combined organic extracts were washed with brine and dried over Na₂SO₄. Removal of the solvent gave the crude product which was then purified on flash chromatography (hexane/EtOAc to CH₂Cl₂/MeOH) to give pure Fmoc-cis-APC (18.2 g, 72% overall yield for two steps) and Fmoc-trans-APC (2.1 g, 8%). Fmoc-15 cis-APC, ^1H NMR(CD₃OD), 7.79 (d, 2H), 7.72 (d, 2H), 7.37 (t, 2), 7.24~7.32 (m, 4), 7.14~7.23 (m, 3), 4.37 (d, 2H), 4.24 (t, 1H), 2.55 (m, 1H), 2.28 (m, 2H), 1.84~1.96 (m, 2H) and 1.64~1.73 (m, 4H).

EXAMPLE 2

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Preparation of Fmoc-1-amino-4-(4-methoxyphenyl)cyclohexane-1-carboxylic acid (Fmoc-4-MeOApC-OH)

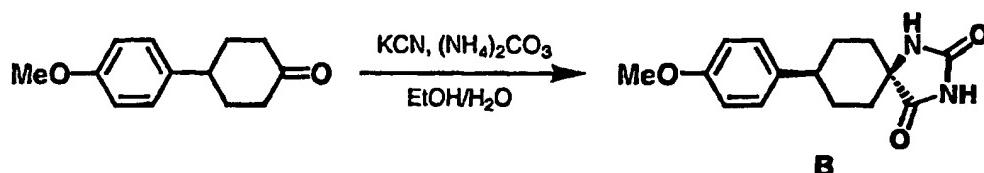
Step 1:



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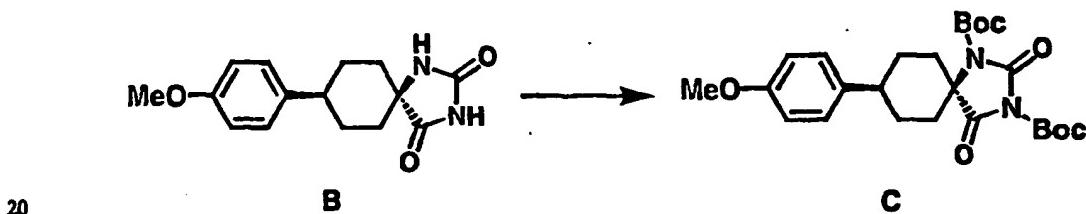
A solution of 4-(4-hydroxyphenyl)cyclohexanone (5.0 g, 26.3 mmol) in acetone (100 mL) was treated with K₂CO₃ (14.5 g, 105 mmol, 4 equiv) and iodomethane (4.9 mL, 11.2 g, 78.6 mmol, 3 equiv.). The reaction was heated at 65 °C overnight. After the solvent was removed, the residue was treated with H₂O and extracted with EtOAc. The organic extracts were 5 combined and washed with brine, dried over Na₂SO₄ and concentrated in vacuum to give the spectroscopically pure 4-(4-methoxyphenyl)-cyclohexanone (5.34 g, 100 %). ¹H NMR(CDCl₃) 7.16 (dt, 2H), 6.87 (dt, 2H), 3.78 (s, 3H), 2.99 (tt, 1H), 2.47-2.53 (m, 4H), 2.20 (m, 2H) and 1.83-1.98 (m, 2H); MS (electrospray) *m/e*, 205 (M+1)⁺, Calcd for C₁₃H₁₆O₂, 204.

10 Step 2:



To a solution of the 4-(4-methoxyphenyl)-cyclohexanone (3.86 g, 18.9 mmol) in ethanol (50 mL) and water (15 mL) in a glass pressure bottle, were added ammonium carbonate (14.5 g, 151 mmol, 8 equiv.) and potassium cyanide (2.0 g, 30.7 mmol, 1.6 equiv.). The mixture was 15 heated at 80-90 °C for 24 hrs. The cooled reaction mixture was added to icy water (300 mL) and stirred vigorously for 30 min. The resulting precipitate was suction filtered, washed thoroughly with water and dried to yield the hydantoin B as a white solid (4.75 g, 91% yield). MS (electrospray) *m/e* 273 (M-H), Calcd for C₁₅H₁₈N₂O₃, 274.

Step 3:

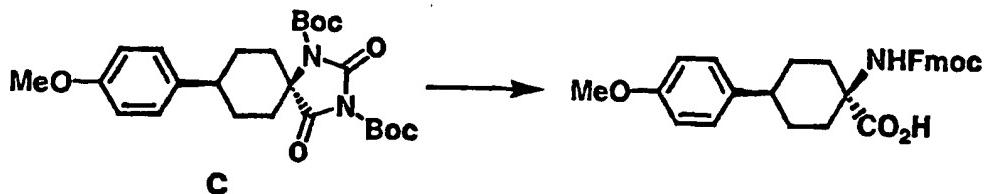


To a suspension of the hydantoin B (18.7 g, 68.25 mmol) in dry THF (450 mL) were added di-*tert*-butyl dicarbonate (37.2 g, 170.5 mmol, 2.5 equiv), triethylamine (10.5 mL, 7.59 g, 75.0 mmol, 1.1 equiv) and DMAP (460 mg, 3.65 mmol) in succession. About 15 minutes after the addition, the reaction turned into a clear yellow solution and was stirred overnight at room 25 temperature. The reaction mixture was concentrated under reduced pressure to yield a solid that

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was then taken up in EtOAc (800 mL), washed with 1N HCl (3x50 mL), saturated aqueous Na₂CO₃ (2x50 mL) and brine (2x50 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude light yellow product was purified through flash chromatography (hexane/EtOAc, 90/10 → 70/30) to give the pure bis-Boc hydantoin C as a white solid (27.6 g, 87%). ¹H NMR (CDCl₃): 7.28 (dt, 2H), 6.88 (dt, 2H), 3.79 (s, 3H), 2.14-2.24 (m, 2H), 1.59 (s, 9H) and 1.38 (s, 9H); MS (electrospray) *m/e* 538 (M+MeCN+Na)⁺, Calcd for C₂₅H₃₄N₂O₇, 474.

Step 4:

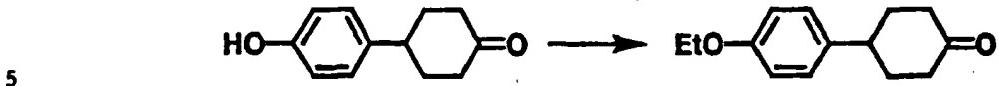


The bis-Boc hydantoin C (15.08 g, 31.78 mmol) was dissolved in DME (500 mL) to give a clear solution. To this solution was added 1N NaOH (290 mL, 290 mmol) and the reaction was stirred overnight at room temperature, giving a slightly cloudy mixture. HPLC showed completion of the reaction. The reaction mixture was concentrated under reduced pressure to remove DME and extracted with Et₂O. Without purification, the resulting aqueous layer containing 1-amino-4-(4-methoxyphenyl)cyclohexane carboxylic acid (4-MeOAPC) was treated with 6N HCl to adjust the pH to 11-12. To this solution (~300 mL) were added DME (300 mL) and a solution of Fmoc-OSu (16.7 g, 49.42 mmol) in DME (200 mL) and the reaction was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to remove DME, acidified with 3N HCl, extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified through flash chromatography (CH₂Cl₂/MeOH, 98/2 → 90/10) to give the pure product Fmoc-4-MeOAPC as a white solid (12.4 g, 83 % yield from the bis-Boc hydantoin C). ¹H NMR (DMSO-d₆), 7.88 (d, 2H), 7.76 (d, 2H), 7.40 (t, 2H), 7.30 (t, 2H), 7.11 (d, 2H), 6.85 (d, 2H), 3.71 (s, 3H); MS (electrospray) *m/e* 470 (M-H), Calcd for C₂₉H₂₉NO₅, 471.

EXAMPLE 3

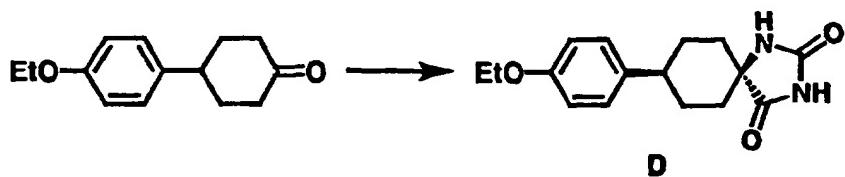
Preparation of Fmoc-1-amino-4-(4-ethoxyphenyl)cyclohexane-1-carboxylic acid (Fmoc-4-EtOApC-OH)

Step 1:



A solution of 4-(4-hydroxyphenyl)cyclohexanone (5.0 g, 26.3 mmol) in acetone (100 mL) was treated with K_2CO_3 (14.5 g, 105 mmol, 4 equiv) and iodoethane (10.5 mL, 20.5 g, 131 mmol, 5 equiv.). The reaction was heated at 65 °C overnight. After the solvent was removed,
 10 the residue was treated with H_2O and extracted with EtOAc. The organic extracts were combined and washed with brine, dried over Na_2SO_4 and concentrated in vacuum to give the spectroscopically pure 4-(4-ethoxyphenyl)-cyclohexanone (5.74 g, 100 %). 1H NMR ($CDCl_3$) 7.15 (dt, 2H), 6.86 (dt, 2H), 4.02 (q, 2H), 2.99 (tt, 1H), 2.46-2.54 (m, 4H), 2.16-2.24 (m, 2H), 1.83-2.00 (m, 2H) and 1.41 (t, 3H); MS (electrospray) m/e , 219 ($M+1$)⁺, Calcd for $C_{14}H_{18}O_2$,
 15 218.

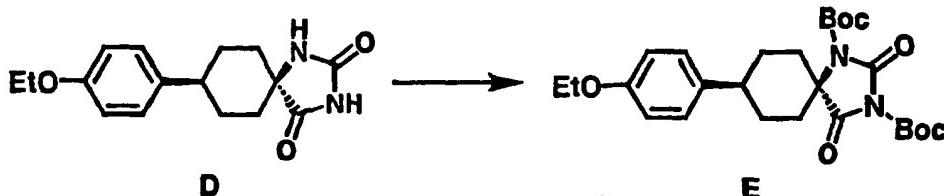
Step 2:



To a solution of the 4-(4-ethoxyphenyl)-cyclohexanone (4.15 g, 19.01 mmol) in ethanol (50 mL) and water (15 mL) in a glass pressure bottle, were added ammonium carbonate (14.5 g, 151 mmol, 8 equiv.) and potassium cyanide (2.05 g, 31.42 mmol, 1.6 equiv.). The mixture was heated at 80-90 °C for 19 hrs. The cooled reaction mixture was added to icy water (300 ml) and stirred vigorously for 30 min. The resulting precipitate was suction filtered, washed thoroughly with water and dried to yield the hydantoin D as a white solid (5.17 g, 94% yield). MS (electrospray) m/e 287 (M-H), Calcd for $C_{16}H_{20}N_2O_3$, 288.

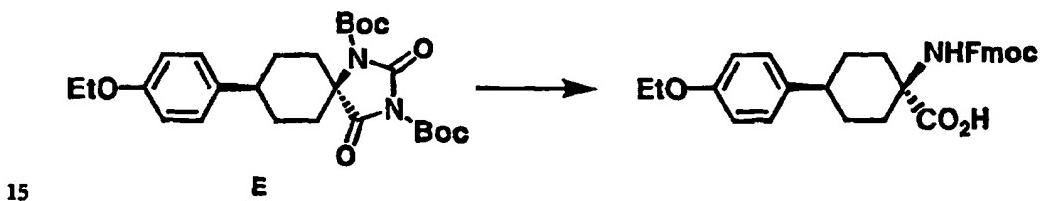
- 69 -

Step 3:



To a suspension of the hydantoin D (4.22 g, 14.65 mmol) in dry THF (100 mL) were added di-*tert*-butyl dicarbonate (7.98 g, 36.60 mmol, 2.5 equiv), triethylamine (2.3 mL, 1.63 g, 16.11 mmol, 1.1 equiv) and DMAP (89.4 mg, 0.73 mmol) in succession. About 15 minutes after the addition, the reaction turned into a clear yellow solution and was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to yield a solid that was then taken up in EtOAc (300 mL), washed with 1N HCl (3x20 mL), saturated aqueous Na₂CO₃ (2x20 mL) and brine (2x20 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude light yellow product was purified through flash chromatography (hexane/EtOAc, 90/10→70/30) to give the pure bis-Boc hydantoin E as a white solid (7.01 g, 98%). ¹H NMR (CDCl₃): 7.27 (dt, 2H), 6.87 (dt, 2H), 4.02 (q, 2H), 1.59 (s, 9H), 1.43 (t, 3H) and 1.38 (s, 9H); MS (electrospray) *m/e* 999 (2M+Na)⁺, Calcd for C₂₆H₃₆N₂O₇, 488.

Step 4:



The bis-Boc hydantoin E (6.58 g, 13.46 mmol) was dissolved in DME (200 mL) to give a clear solution. To this solution was added 1N NaOH (121 mL, 121 mmol) and the reaction was stirred overnight at room temperature, giving a slightly cloudy mixture. HPLC showed completion of the reaction. The reaction mixture was concentrated under reduced pressure to remove DME and extracted with Et₂O. Without purification, the resulting aqueous layer containing 1-amino-4-(4-ethoxyphenyl)cyclohexane carboxylic acid (4-EtOAPC) was treated with 6N HCl to adjust the pH to 11-12. To this solution (~130 mL) were added DME (100 mL) and a solution of Fmoc-OSu (6.83 g, 20.24 mmol) in DME (30 mL) and the reaction was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to remove DME, acidified with 3N HCl, extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The crude product was

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purified through flash chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 98/2 → 90/10) to give the pure product Fmoc-4-EtOAPC as a white solid (5.56g, 85 % yield from the bis-Boc hydantoin E). ^1H NMR (DMSO-d₆), 7.88 (d, 2H), 7.74 (d, 2H), 7.40 (td, 2H), 7.30 (td, 2H), 7.11 (d, 2H), 6.84 (d, 2H), 3.97 (q, 2H) and 1.29 (t, 3H); MS (electrospray) *m/e* 484 (M-H), Calcd for C₃₀H₃₁NO₅, 485.

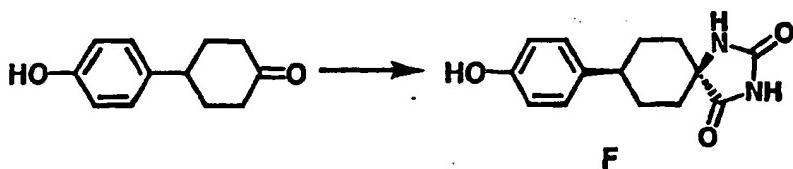
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EXAMPLE 4

Preparation of Fmoc-1-amino-4-(4-hydroxyphenyl)cyclohexane-1-carboxylic acid (Fmoc-4-HOApC-OH)

Step 1:

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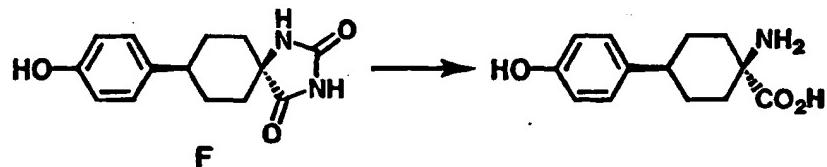


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To a solution of 4-(4-hydroxyphenyl)cyclohexanone (2.00 g, 10.52 mmol) in ethanol (30 mL) and water (10 mL) in a glass pressure bottle, were added ammonium carbonate (6.17 g, 64.2 mmol, 6 equiv.) and potassium cyanide (1.07 g, 15.8 mmol, 1.5 equiv.). The mixture was heated at 80-90 °C overnight. The cooled reaction mixture was added to icy water (200 ml) and stirred vigorously for 30 min. The resulting precipitate was suction filtered, washed thoroughly with water and dried to yield the hydantoin F as a white solid (2.56 g, 94% yield). MS (electrospray) *m/e* 261 (M+H)⁺, Calcd for C₁₄H₁₈N₂O₃, 260.

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Step 2:



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The hydantoin F (2.10 g, 8.06 mmol) was suspended in aqueous NaOH (6N, 100 mL) and heated at 130 °C for 2-3 days. Upon the completion of the hydrolysis, the reaction mixture was neutralized with conc. HCl to slightly acidic (pH~6). The resulting slurry was filtered, washed with water and dried to give 1-amino-4-(4-hydroxyphenyl)cyclohexane carboxylic acid

- 71 -

(4-HOAPC) as a white solid (3.1 g, >100 % yield. contaminated with inorganic salt). MS (electrospray) m/e 236 ($M+H$)⁺, Calcd for $C_{13}H_{17}NO_3$, 235.

Step 3:



5 The crude 1-amino-4-(4-hydroxyphenyl)cyclohexane carboxylic acid (4-HOAPC) from the last step (3.1 g) was suspended in dioxane (100 mL) and aqueous 10 % Na_2CO_3 (50 mL) and stirred vigorously overnight. The reaction mixture was concentrated to remove dioxane, neutralized with 6N HCl to slightly acidic (pH 5-6) and extracted with EtOAc. The combined organic extracts were washed with brine and dried over Na_2SO_4 . Removal of the solvent gave the
 10 crude product which was purified on flash chromatography (hexane/EtOAc to $CH_2Cl_2/MeOH$) to give pure Fmoc-4-HOAPC (2.76 g, 75% overall yield for two steps). 1H NMR(CD_3OD), 7.78 (d, 2H), 7.72 (d, 2H), 7.38 (t, 2H), 7.30 (td, 2H), 7.04 (d, 2H), 6.72 (dt, 2H), 4.38 (d, 2H), 4.25 (t, 1H), 2.46 (m, 1H), 2.24-2.34 (m, 2H) and 1.81-1.92 (m, 6H); MS (electrospray) m/e 456 ($M-H$), Calcd for $C_{28}H_{27}NO_5$, 457.

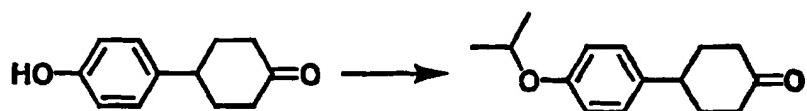
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EXAMPLE 5

Preparation of Fmoc-1-amino-4-(4-isopropoxypyhenyl)cyclohexane-1-carboxylic acid (Fmoc-4-iPrOApC-OH)

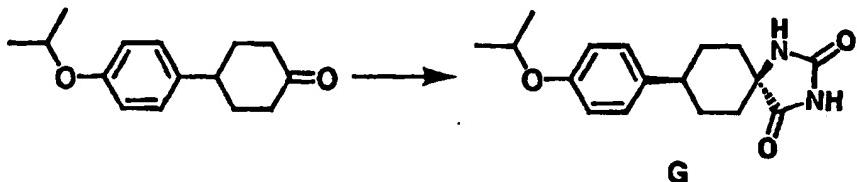
Step 1:

20



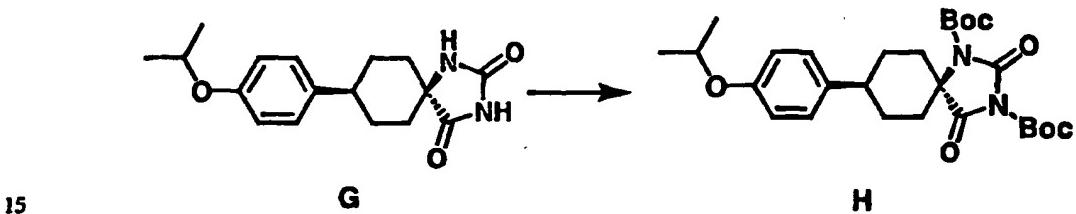
A solution of 4-(4-hydroxyphenyl)cyclohexanone (6.0 g, 31.6 mmol) in DMF (90 mL) was treated with K_2CO_3 (21 g, 158 mmol, 5 equiv) and 2-iodopropane (15 mL, 26.8 g, 158 mmol, 5 equiv.). The reaction was heated at 100 °C overnight. After the solvent was removed,
 25 the residue was treated with H_2O and extracted with EtOAc. The organic extracts were combined and washed with brine, dried over Na_2SO_4 and concentrated in vacuum to give the spectroscopically pure 4-(4-isopropoxypyhenyl)cyclohexanone (7.02 g, 95 %). 1H NMR ($CDCl_3$): 7.14 (dt, 2H), 6.84 (dt, 2H), 4.3 (septet, 1H), 2.97 (tt, 1H), 2.46-2.52 (m, 4H), 2.16-2.24 (m, 2H), 1.83-1.98 (m, 2H) and 1.33 (d, 6H).

Step 2:



To a solution of the 4-(4-isopropoxyphenyl)cyclohexanone (5.1 g, 21.98 mmol) in
 5 ethanol (90 mL) and water (30 mL) in a glass pressure bottle, were added ammonium carbonate
 (12.6 g, 131 mmol, 6 equiv.) and potassium cyanide (2.14 g, 32.9 mmol, 1.5 equiv.). The mixture
 was heated at 80-90 °C for 24 hrs. The cooled reaction mixture was added to icy water (400 ml)
 and stirred vigorously for 30 min. The resulting precipitate was suction filtered, washed
 thoroughly with water and dried to yield hydantoin G as a white solid (6.60 g, 99% yield).
 10 ^1H NMR (DMSO-d₆): 10.60 (s, 1H), 8.65 (s, 1H), 7.18 (d, 2H), 6.80 (d, 2H), 4.52 (septet, 1H), 2.43
 (m, 1H), 1.85-2.15 (m, 2H), 1.56-1.80 (m, 6H) and 1.22 (d, 6H); MS (electrospray) *m/e* 301 (M-
 H), Calcd for C₁₇H₂₂N₂O₃, 302.

Step 3:

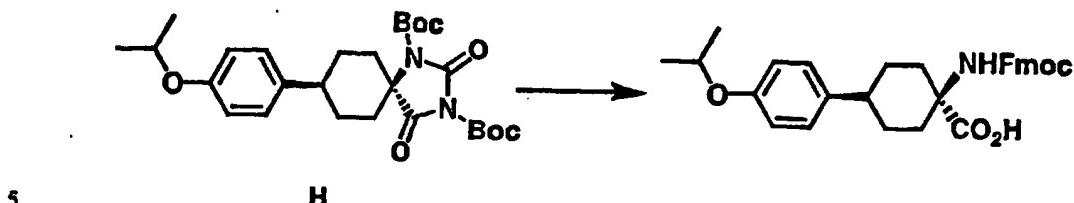


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To a suspension of the hydantoin G (5.8 g, 19.20 mmol) in dry THF (180 mL) were
 added di-*tert*-butyl dicarbonate (10.46 g, 48.0 mmol, 2.5 equiv), triethylamine (2.9 mL, 2.13 g,
 21.12 mmol, 1.1 equiv) and DMAP (140 mg, 1.15 mmol) in succession. About 15 minutes after
 the addition, the reaction turned into a clear yellow solution and was stirred overnight at room
 20 temperature. The reaction mixture was concentrated under reduced pressure to yield a solid that
 was then taken up in EtOAc (600 mL), washed with 1N HCl (3x40 mL), saturated aqueous
 Na₂CO₃ (2x40 mL) and brine (2x40 mL), dried over anhydrous Na₂SO₄ and concentrated under
 reduced pressure. The crude light yellow product was purified through flash chromatography
 (hexane/EtOAc, 90/10 → 80/20) to give the pure bis-Boc hydantoin H as a white solid (9.4 g,
 25 98%). ^1H NMR (CDCl₃): 7.27 (dt, 2H), 6.87 (dt, 2H), 4.02 (q, 2H), 2.98 (t, 1H), 2.26-2.56 (m,

- 73 -

4H), 2.14-2.24 (m, 2H), 1.76-1.86 (m, 2H), 1.59 (s, 9H), 1.43 (t, 3H) and 1.38 (s, 9H); MS (electrospray) m/e 999 ($2M+Na$)⁺, Calcd for $C_{26}H_{36}N_2O_7$, 488.

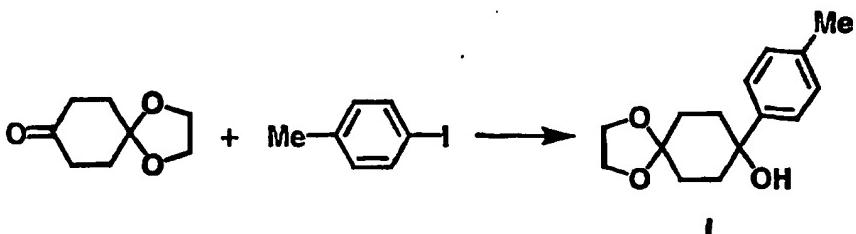
Step 4:

- The bis-Boc hydantoin H (4.34 g, 8.64 mmol) was dissolved in DME (100 mL) to give a clear solution. To this solution was added 1N NaOH (78 mL, 78 mmol) and the reaction was stirred overnight at room temperature, giving a fairly clear mixture. HPLC showed completion of the reaction. The reaction mixture was concentrated under reduced pressure to remove DME and extracted with Et₂O. Without purification, the resulting aqueous layer containing 1-amino-4-(4-isopropoxyphenyl)cyclohexane carboxylic acid (4-iPrOAPC) was treated with 6N HCl to adjust the pH to 11-12. To this solution (~90 mL) were added DME (120 mL) and a solution of Fmoc-OSu (3.49 g, 10.34 mmol, 1.2 equiv) in DME (20 mL) and the reaction was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to remove DME, acidified with 3N HCl, extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified through flash chromatography (hexane/EtOAc → CH₂Cl₂/MeOH) to give the pure product Fmoc-4-iPrOAPC as a white solid (3.23, 75 % yield from bis-Boc hydantoin H). ¹H NMR(DMSO-d₆), 7.76 (d, 2H), 7.60 (d, 2H), 7.39 (t, 2H), 7.31 (t, 2H), 7.08 (d, 2H), 6.84 (d, 2H), 4.24 (m, 1H) and 1.34 (d, 6H); MS (electrospray) m/e 498 (M-H), Calcd for $C_{31}H_{33}NO_5$, 499.

EXAMPLE 6

Preparation of Fmoc-1-amino-4-(4-methylphenyl)cyclohexane-1-carboxylic acid (Fmoc-4-MeApc-OH)

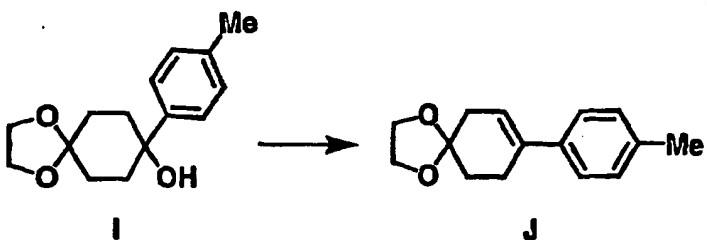
Step 1:



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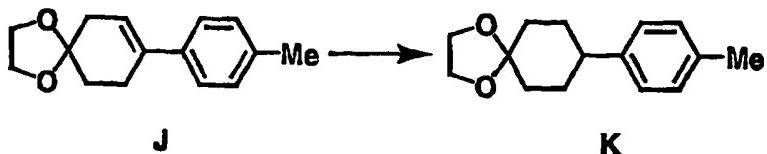
To a solution of 4-iodotoluene (10.9 g, 50.0 mmol) in dry THF (180 mL) at -78 °C was added a solution of n-BuLi (1.6 M, 31.0 mL, 50 mmol) in hexane over 20 min. The reaction was stirred for another 20 min before a solution of 1,4-cyclohexanedione *mono*-ethylene ketal (6.0 g, 38.46 mmol) in dry THF (100 mL) was added dropwise. After stirred for 2 h at -78 °C, the reaction was quenched with aqueous NH₄Cl and extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄, concentrated in vacuo to give the spectroscopically pure product I as a white solid (9.34 g, 98% yield). ¹H NMR (CDCl₃): 7.41 (m, 2H), 7.16 (d, 2H), 3.98 (m, 4H), 2.34 (s, 3H); MS (EI) *m/e* 248 (M⁺), Calcd for C₁₅H₂₀O₃, 248.

15 Step 2:

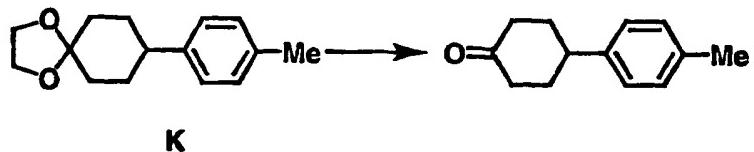


To a solution of the alcohol I (9.10g, 36.65 mmol) in dry benzene (200 mL) in a flask equipped with a Dean-Stark trap, was added p-toluenesulfonic acid monohydrate (650 mg) and the reaction was heated at 100 °C for 3 hrs. The reaction was cooled to rt, diluted with EtOAc (500 mL) and washed with aqueous Na₂CO₃ (50 mL), brine (3x50 mL), dried over Na₂SO₄ and concentrated under reduced pressure to give the spectroscopically pure product J (8.36 g, 100 yield), which was used for next step without purification. MS (EI) *m/e* 230 (M⁺), 190 (M-OCH₂CH₂O), Calcd for C₁₅H₁₈O₂, 230.

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Step 3:

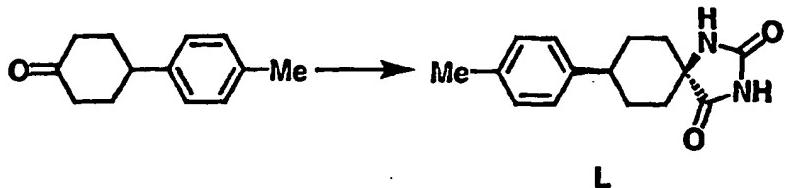
To a solution of the olefin J (7.49 g) in EtOAc (180 mL) was added Pd/C (5 wt % on carbon, 800 mg) and the reaction was run under 40 psi of hydrogen for 3 hrs at room temperature. The catalyst was filtered off and the filtrate was concentrated to give the spectroscopically pure product K as a colorless oil (7.40 g, 100% yield). MS (EI) m/e 232 (M^+), 188 (M-OCH₂CH₂). Calcd for C₁₅H₂₀O₂, 232.

10 Step 4:

A solution of the ketal K (6.90 g) in acetone (140 mL) was treated with 4N HCl (60 mL) and heated at 65 °C for 4 hrs. Solvent was removed and the residue was diluted with EtOAc and neutralized with 4N HCl. The aqueous was extracted with EtOAc. The combined organic extracts were washed with brine, dried and concentrated. The resulting crude 4-(4-methylphenyl)cyclohexanone was used for next step without purification (5.57 g, quantitative yield). MS (EI) m/e 188 (M^+), Calcd for C₁₃H₁₆O, 188.

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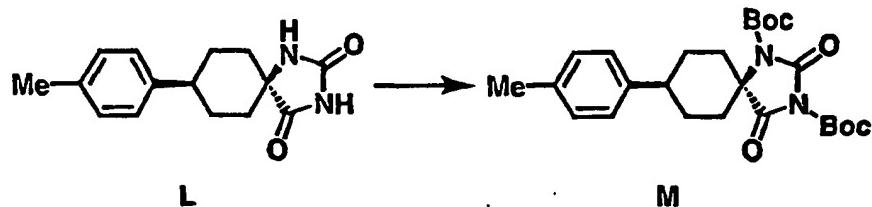
Step 5:



- To a solution of 4-(4-methylphenyl)cyclohexanone (5.32 g, 28.3 mmol) in ethanol (90 mL) and water (30 mL) in a glass pressure bottle, were added ammonium carbonate (16.3 g, 169.8 mmol, 6 equiv.) and potassium cyanide (3.68 g, 56.5 mmol, 2 equiv.). The mixture was heated at 80-90 °C overnight. The cooled reaction mixture was added to icy water (400 ml) and stirred vigorously for 30 min. The resulting precipitate was suction filtered, washed thoroughly with water and dried to yield the hydantoin L as a white solid (6.3 g, 86% yield). MS (electrospray) *m/e* 517 (2M+H), Calcd for C₁₅H₁₈ClN₂O₂, 258

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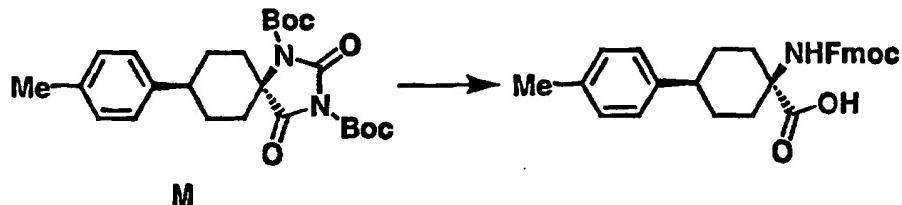
Step 6:



- To a suspension of the hydantoin L (5.82 g, 22.55 mmol) in dry THF (250 mL) were added di-*tert*-butyl dicarbonate (12.3 g, 56.4 mmol, 2.5 equiv), triethylamine (3.5 mL, 2.5 g, 24.7 mmol, 1.1 equiv) and DMAP (275 mg, 2.25 mmol) in succession. The reaction turned into a clear yellow solution and was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to yield a solid that was then taken up in EtOAc (500 mL), washed with 1N HCl (3x50 mL), saturated aqueous Na₂CO₃ (2x50 mL) and brine (2x50 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude light yellow product was purified through flash chromatography (hexane/EtOAc, 90/10→70/30) to give the pure bis-Boc hydantoin M as a white solid (10.03 g, 100% yield). ¹H NMR (CDCl₃): 7.26 (d, 2H), 6.87 (d, 2H), 3.00 (m, 1H), 2.32 (s, 3H), 1.59 (s, 9H) and 1.37 (s, 9H).

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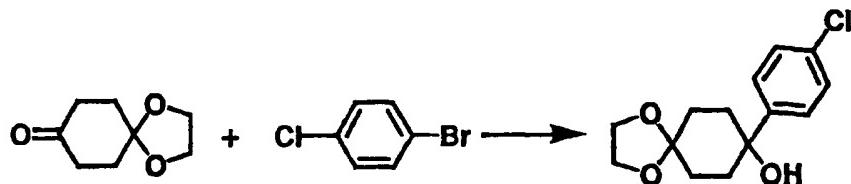
Step 7:



The bis-Boc hydantoin M (6.40 g, 13.97 mmol) was dissolved in DME (200 mL) to give a clear solution. To this solution was added 1N NaOH (120 mL, 120 mmol) and the reaction was stirred overnight at room temperature, giving a slightly cloudy mixture. HPLC showed completion of the reaction. The reaction mixture was concentrated under reduced pressure to remove DME and extracted with Et₂O. Without purification, the resulting aqueous layer containing 1-amino-4-(4-methylphenyl)cyclohexane carboxylic acid (4-MeAPC) was treated with 6N HCl to adjust the pH to 11-12. To this solution (~140 mL) were added DME (240 mL) and a solution of Fmoc-OSu (5.10 g, 15.13 mmol, 1.1 equiv) in DME (40 mL) and the reaction was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to remove DME, acidified with 3N HCl, extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified through flash chromatography (CH₂Cl₂/MeOH, 98/2 → 90/10) to give the pure product Fmoc-4-MeAPC as a white solid (4.35 g, 69 % yield from bis-Boc hydantoin M). ¹H NMR (DMSO-d₆): 7.88 (d, 2H), 7.75 (d, 2H), 7.24-7.43 (m, 4H), 7.02-7.14 (m, 4H), 4.25 (m, 3H), 2.24 (s, 3H).

EXAMPLE 7

- 20 Preparation of Fmoc-1-amino-4-(4-chlorophenyl)cyclohexane-1-carboxylic acid (Fmoc-4-ClApc-OH)



Step 1:

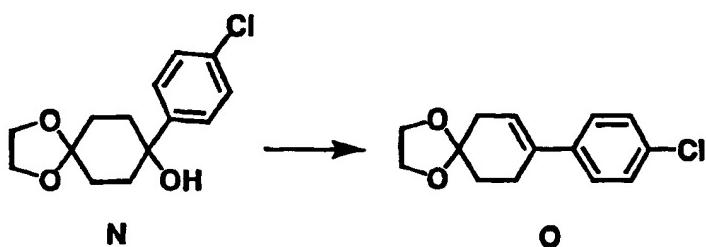
A solution of 4-chlorophenylbromide (7.5 g, 39.2 mmol) in dry THF (180 mL) was cooled to -78 °C and treated dropwise with a solution of n-BuLi (1.6 M, 25 mL, 40 mmol) in hexane over 20 min. The reaction was stirred for a further 30 min before a solution of 1,4-

- 78 -

cyclohexanedione mono-ethylene ketal (6.0 g, 38.46 mmol) in dry THF (100 mL) was added dropwise. After stirred for 1 hr at -78 °C, the reaction was quenched with aqueous NH₄Cl and extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄, concentrated in vacuo to give the spectroscopically pure product N as a white solid (9.40 g, 91% yield). ¹H NMR (CDCl₃): 7.45 (m 2H), 7.31 (m, 2H), 3.99 (m, 4H), 2.02-2.20 (m, 4H), 1.75-1.82 (m, 2H), 1.66-1.73 (m, 2H), 1.54 (s, 1H); MS (EI) *m/e* 268 (M⁺), 251 (M-OH), 250 (M-H₂O), Calcd for C₁₄H₁₇ClO₃, 268.

Step 2:

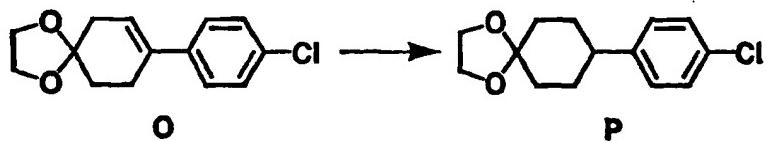
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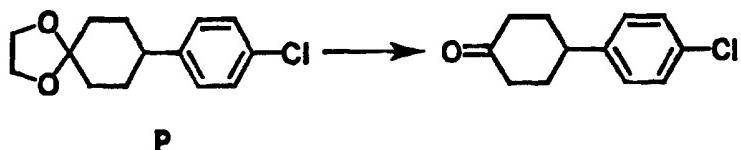
To a solution of the alcohol N (6.78 g, 25.30 mmol) in dry benzene (120 mL) in a flask equipped with a Dean-Stark trap, was added p-toluenesulfonic acid monohydrate (960 mg) and the reaction was heated at reflux for 3 hrs. The reaction was cooled to rt, diluted with EtOAc (500 mL) and washed with aqueous Na₂CO₃ (50 mL), brine (3x50 mL), dried over Na₂SO₄ and 15 concentrated under reduced pressure to give the spectroscopically pure product O (6.30 g, 100 yield), which was used for next step without purification. MS (EI) *m/e* 250 (M⁺), 190 (M-OCH₂CH₂O), Calcd for C₁₄H₁₅ClO₂, 250.

Step 3:

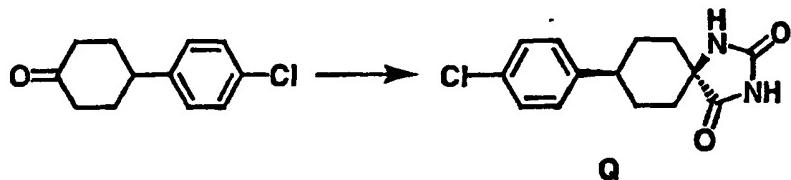
20



To a solution of the olefin O (6.11 g) in EtOAc (120 mL) was added Pd/C (5 wt % on carbon, 600 mg) and the reaction was run under 5 psi of hydrogen for 3 hrs at room temperature. The catalyst was filtered off and the filtrate was concentrated to give the 25 spectroscopically pure product P as a colorless oil (6.10 g, 100% yield). MS (EI) *m/e* 252(M⁺), Calcd for C₁₄H₁₇ClO₂, 252.

Step 4:

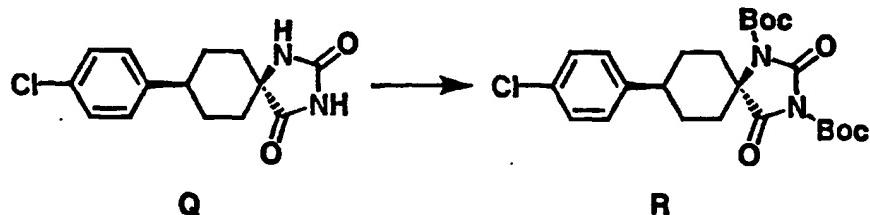
- 5 A solution of the ketal P (5.81 g, 23.06 mmol) in acetone (200 mL) was treated with p-toluenesulfonic acid monohydrate (876 mg) and heated at 60 °C overnight. Solvent was removed and the residue was taken up in EtOAc, washed with aqueous Na₂CO₃ solution, brine, dried and concentrated to give the crude product as a yellow oil (5.38 g, >100% yield). Purification through flash chromatography (hexane/EtOAc, 80/20 → 60/40) provided 4-(4-chlorophenyl)cyclohexanone as a light yellow oil (4.54 g, 95% yield). MS (EI) *m/e* 208 (M⁺), Calcd for C₁₂H₁₃ClO₂, 208.

Step 5:

- 15 To a solution of 4-(4-chlorophenyl)cyclohexanone (4.26 g, 20.48 mmol) in ethanol (90 mL) and water (30 mL) in a glass pressure bottle, were added ammonium carbonate (13.8 g, 144 mmol, 7 equiv) and potassium cyanide (3.56 g, 54.77 mmol, 2.5 equiv). The mixture was heated at 80-90 °C overnight. The cooled reaction mixture was added to icy water (400 ml) and stirred vigorously for 30 min. The resulting precipitate was suction filtered, washed thoroughly with water and dried to yield the hydantoin Q as a white solid (5.58 g, 98% yield). MS (electrospray) *m/e* 277 (M-H), Calcd for C₁₄H₁₅ClN₂O₂, 278

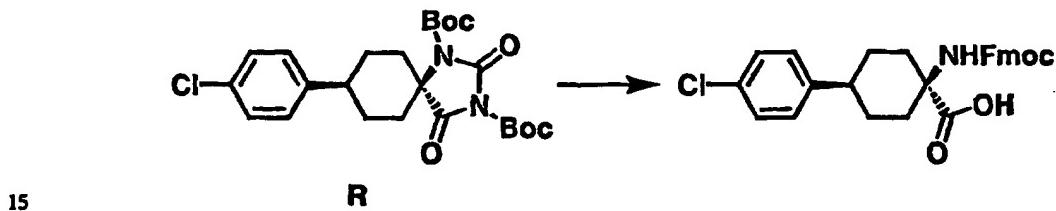
- 80 -

Step 6:



To a suspension of the hydantoin **Q** (5.15 g, 18.5 mmol) in dry THF (250 mL) were added di-*tert*-butyl dicarbonate (10.1 g, 46.3 mmol, 2.5 equiv), triethylamine (2.8 mL, 2.07 g, 5 20.45 mmol, 1.1 equiv) and DMAP (226 mg, 1.85 mmol) in succession. The reaction turned into a clear yellow solution and was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to yield a solid that was then taken up in EtOAc (500 mL), washed with 1N HCl (3x50 mL), saturated aqueous Na₂CO₃ (2x50 mL) and brine (2x50 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude light yellow 10 product was purified through flash chromatography (hexane/EtOAc, 90/10→70/30) to give the pure bis-Boc hydantoin **R** as a white solid (8.05 g, 91% yield). MS (electrospray) *m/e* 542 (M+Ma+MeCN), Calcd for C₂₄H₃₁CIN₂O₆, 478

Step 7:



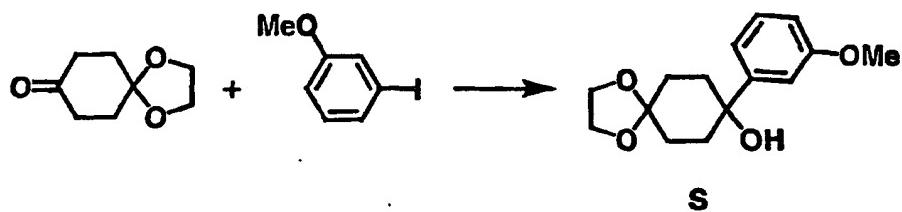
The bis-Boc hydantoin **R** (6.41 g, 13.97 mmol) was dissolved in DME (200 mL) to give a clear solution. To this solution was added 1N NaOH (120 mL, 120 mmol) and the reaction was stirred overnight at room temperature, giving a slightly cloudy mixture. HPLC showed completion of the reaction. The reaction mixture was concentrated under reduced pressure to 20 remove DME and extracted with Et₂O. Without purification, the resulting aqueous layer containing 1-amino-4-(4-chlorophenyl)cyclohexane carboxylic acid (4-ClAPC) was treated with 6N HCl to adjust the pH to 11-12. To this solution (~180 mL) were added DME (240 mL) and a solution of Fmoc-OSu (5.31 g, 15.74 mmol, 1.1 equiv) in DME (30 mL) and the reaction was stirred overnight at room temperature. The reaction mixture was concentrated under reduced 25 pressure to remove DME, acidified with 3N HCl, extracted with EtOAc. The combined organic

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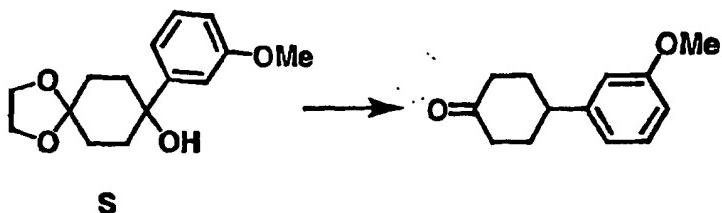
extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified through flash chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 98/2 → 90/10) to give the pure product Fmoc-4-ClAPC as a white solid (5.04 g, 76% yield from the bis-Boc hydantoin). ^1H NMR (DMSO-d_6), 7.88 (d, 2H), 7.74 (d, 2H), 7.19-7.42 (m, 8H), 4.20-4.31 (m, 3H); MS (electrospray) m/e 474 (M-H), Calcd for $\text{C}_{28}\text{H}_{26}\text{ClNO}_4$, 475.

EXAMPLE 8

Preparation of Fmoc-1-amino-4-(3-methoxyphenyl)cyclohexane-1-carboxylic acid (Fmoc-3-MeOApC-OH)

10 **Step 1:**

To a solution of 3-iodoanisole (11.7, 50.0 mmol, 1.3 equiv) in dry THF (180 mL) at -78 °C was added a solution of n-BuLi (1.6 M, 31.0 mL, 50 mmol, 1.3 equiv) in hexane over 25 min. 15 The reaction was stirred for another 30 min before a solution of 1,4-cyclohexanedione mono-ethylene ketal (6.0 g, 38.46 mmol) in dry THF (100 mL) was added dropwise. After stirred for 2 h at -78 °C, the reaction was quenched with aqueous NH_4Cl and extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na_2SO_4 , concentrated in vacuo to give the spectroscopically pure product S as a white solid (9.34 g, 98% yield). ^1H NMR (CDCl_3): 20 7.26 (dd, 1H), 7.06-7.11 (m, 2H), 6.79 (dd, 1H), 3.98 (m, 4H), 3.81 (s, 3H).

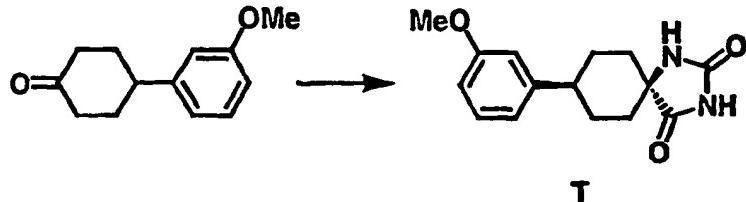
Step 2:

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To a stirred solution of the alcohol S (5.6 g, 21.21 mmol) in dry CH_2Cl_2 (200 mL) under a nitrogen atmosphere at salt-ice bath temperature, were added in succession triethylsilane (10.2 mL, 7.4 g, 63.63 mmol, 3 equiv) and boron trifluoride etherate (21.5 mL, 24.1 g, 169.7 mmol, 8 equiv). The reaction mixture was then allowed to warm to room temperature and stirred for 3 hrs before washed with 10% aqueous K_2CO_3 solution and H_2O , dried over Na_2SO_4 , and concentrated in vacuo to give the deoxygenation compound as an oil (4.91g), which was sufficiently pure for direct use. This crude intermediate was dissolved in acetone (130 mL) and treated with 4N HCl (60 mL) and heated at 65 °C for 4 hrs. Solvent was removed under reduced pressure and the residue was diluted with EtOAc and neutralized with 4N NaOH solution. The aqueous layer was extracted with EtOAc and the combined organic extracts were washed with brine, dried and concentrated. The resulting residue was purified by flash chromatography on silica gel (80/20 → 60/40) to give 4-(3-methoxyphenyl)cyclohexanone (3.67 g, 85% overall yield) as a yellow oil. ^1H NMR (CDCl_3): 7.25 (dt, 1H), 6.75-6.86 (m, 3H), 3.81 (s, 3H), 3.00 (t, 1H); MS (EI) m/e 204 (M $^+$), Calcd for $\text{C}_{13}\text{H}_{16}\text{O}_2$, 204.

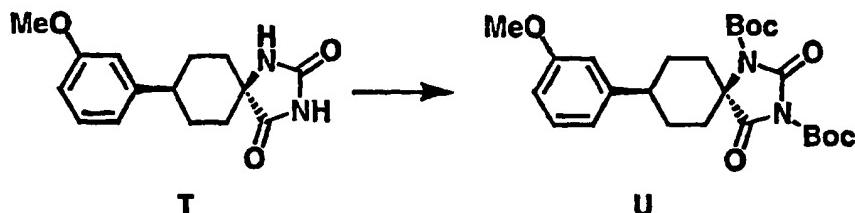
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Step 3:



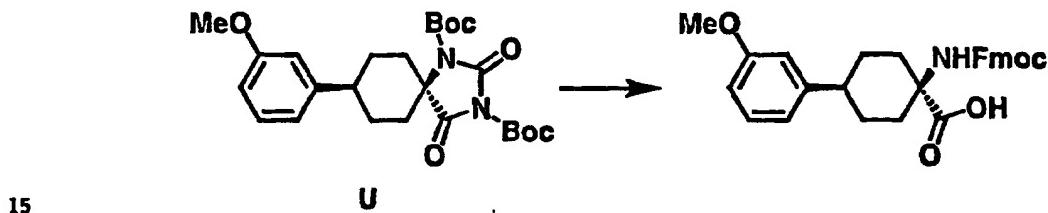
To a solution of 4-(3-methoxyphenyl)cyclohexanone (3.10 g, 15.20 mmol) in ethanol (60 mL) and water (20 mL) in a glass pressure bottle, were added ammonium carbonate (8.75 g, 91.20 mmol, 6 equiv.) and potassium cyanide (1.98 g, 30.40 mmol, 2 equiv.). The mixture was heated at 80-90 °C overnight. The cooled reaction mixture was added to icy water (300 mL) and stirred vigorously for 30 min. The resulting precipitate was suction filtered, washed thoroughly with water and dried to yield the hydantoin T as a white solid (4.08 g, 98% yield). ^1H NMR (DMSO-d₆): 7.11 (d, 1H), 6.70-6.94 (m, 3H), 3.72 (s, 3H); MS (electrospray) m/e 316 (M+MeCN+H), Calcd for $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_3$, 274.

Step 4:



To a suspension of the hydantoin T (5.29 g, 19.30 mmol) in dry THF (250 mL) were
 5 added di-*tert*-butyl dicarbonate (10.5 g, 48.16 mmol, 2.5 equiv), triethylamine (3.0 mL, 2.17 g,
 21.52 mmol, 1.1 equiv) and DMAP (235 mg, 1.92 mmol) in succession. The reaction turned into
 a clear yellow solution and was stirred overnight at room temperature. The reaction mixture was
 concentrated under reduced pressure to yield a solid that was then taken up in EtOAc (500 mL),
 washed with 1N HCl (3x50 mL), saturated aqueous Na₂CO₃ (2x50 mL) and brine (2x50 mL),
 10 dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude light yellow
 product was purified through flash chromatography (hexane/EtOAc, 80/20→60/40) to give the
 pure bis-Boc hydantoin U as a white solid (8.70 g, 95% yield). MS (electrospray) *m/e* 538
 (M+MeCN+Na), Calcd for C₂₅H₃₄N₂O₇, 474.

Step 5:



The bis-Boc hydantoin U (2.30 g, 4.84 mmol) was dissolved in DME (80 mL) to give a
 clear solution. To this solution was added 1N NaOH (44 mL, 44 mmol) and the reaction was
 stirred overnight at room temperature, giving a slightly cloudy mixture. HPLC showed
 completion of the reaction. The reaction mixture was concentrated under reduced pressure to
 20 remove DME and extracted with Et₂O. Without purification, the resulting aqueous layer
 containing 1-amino-4-(3-methoxyphenyl)cyclohexane carboxylic acid (3-MeOAPC) was treated
 with 6N HCl to adjust the pH to 11-12. To this solution (~40 mL) were added dioxane (80 mL)
 and Fmoc-Cl (1.73 g, 6.76 mmol, 1.4 equiv) and the reaction was stirred overnight at room
 temperature. The reaction mixture was then concentrated under reduced pressure to remove
 25 DME, neutralized with 3N HCl and extracted with EtOAc. The combined organic extracts were

washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by flash chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 98/2 → 90/10) to give Fmoc-3-MeOAPC as a white solid (1.98 g, 87 % yield from bis-Boc hydantoin U). ^1H NMR (DMSO-d_6), 7.88 (d, 2H), 7.75 (d, 2H), 7.40 (td, 2H), 7.30 (td, 2H), 7.21 (m, 1H), 6.71-6.80 (m, 3H), 3.72 (s, 3H); MS (electrospray) m/e 494 ($\text{M}+\text{Na}$), Calcd for $\text{C}_{29}\text{H}_{29}\text{NO}_5$, 471.

5 3H); MS (electrospray) m/e 494 ($\text{M}+\text{Na}$), Calcd for $\text{C}_{29}\text{H}_{29}\text{NO}_5$, 471.

EXAMPLE 28

Preparation of Fmoc-(D,L)-5-bromo-2 aminotetraline-2-carboxylic acid (Fmoc-(D,L) 5-Br-Atc-OH)

10

Step 1:



A mixture of 3-(2-bromophenyl)propanoic acid (prepared in 2 steps from 2-bromobenzyl bromide, 2.0 g, 8.73 mmole), oxalyl chloride (1.14 ml, 13.1 mmole) and methylene chloride (20 ml) was cooled in an ice bath and N,N -dimethylformamide (34 μL , 0.44 mmole) was added dropwise. The mixture was stirred at room temperature for 3 hours. Concentration in vacuo gave 3-(2-bromophenyl)propanoyl chloride which was taken up in methylene chloride and used in the next step as a crude.

15

Step 2:



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A solution of the above acid chloride (crude, 8.73 mmole) in methylene chloride was slowly added to a solution of diazomethane (generated from 5.70 g of 1-methyl-3-nitro-1-nitrosoguanidine) in ether (40 ml) cooled in an ice bath. The mixture was then warmed up to room temperature and stirred overnight. The mixture was concentrated in vacuo and purified by column chromatography (10 → 20% ethyl acetate/hexanes) to give 1-diazo-4-(2-bromophenyl)butan-2-one (1.88 g, 85% over 2 steps). ^1H NMR (CDCl_3) δ 7.50 (1H, d, phenyl),

25

- 85 -

7.24 (2H, m, phenyl), 7.06 (1H, m, phenyl), 5.21 (1H, broad s, diazo), 3.05 (2H, t, benzylic), 2.62 (2H, m).

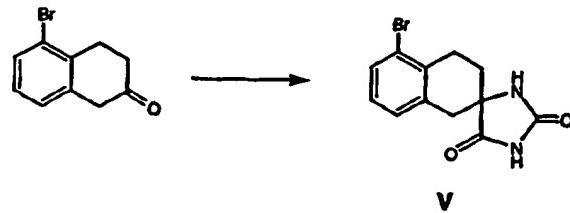
Step 3:

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- To a mixture of rhodium (II) acetate dimer (15 mg, 0.068 mmole) in methylene chloride (120 ml) under reflux was slowly added a solution of 1-diazo-4-(2-bromophenyl)butan-2-one (1.74 g, 6.85 mmole) in methylene chloride (30 ml). After the addition was complete, the mixture was refluxed for an extra twenty minutes. The mixture was cooled to room temperature, trifluoroacetic acid (1.5 ml) was added and the mixture was stirred at room temperature for an hour. The reaction was quenched with saturated sodium bicarbonate solution. The layers were separated and the methylene chloride layer was washed once more with saturated sodium bicarbonate solution. The combined aqueous layers were back-extracted with methylene chloride. The combined organic layers were dried over magnesium sulfate, filtered and concentrated in vacuo to give a brown oil. Purification by column chromatography (10 → 15% ethyl acetate/hexanes) gave 5-bromo- β -tetralone (1.18 g, 77% yield) as a colorless oil. 1H NMR ($CDCl_3$) δ 7.46 (1H, t, phenyl), 7.05-7.09 (2H, m, phenyl), 3.58 (2H, s, benzylic), 3.22 (2H, t, benzylic), 2.54 (2H, t).

Step 4:



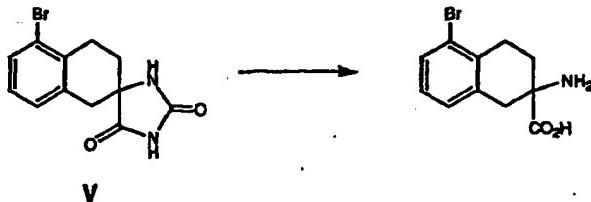
- A mixture of 5-bromo- β -tetralone (1.18 g, 5.24 mmole), potassium cyanide (512 mg, 7.86 mmole), ammonium carbonate (3.0 g, 31.22 mmole), ethanol (25 ml) and water (5 ml) in a sealed, thick walled pressure flask was heated in a 80°C oil bath for 4 days. After cooling to room temperature, the white slurry was poured into ice-water and stirred at room temperature for a

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couple of hours. Filtration followed by air-drying gave hydantoin V (1.31 g, 85%). ¹H NMR (DMSO-d₆) δ 10.71 (1H, broad, NH), 8.28 (1H, broad s, NH), 7.0-7.5 (3H, m, phenyl). LRMS (Electrospray): C₁₂H₁₁BrN₂O₂, calc. 294; observed: 293 (M-H), 295 (M-H).

Step 5:

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A mixture of hydantoin V (1.287 g, 4.36 mmole), Ba(OH)₂·H₂O (4.20 g, 22.2 mmole) in water (25 ml) in a sealed, thick walled pressure flask was heated in a 125°C oil bath for 4 days. The reaction mixture was cooled to room temperature, acidified to ~ pH 3 using 4N sulfuric acid 10 while being stirred vigorously. The suspension was stirred in a boiling water bath for one hour and cooled to room temperature. The white suspension was filtered and the precipitates rinsed with water. The combined filtrate and washings were concentrated in vacuo to ~ 20 ml. Neutralization with concentrated ammonium hydroxide solution gave white precipitate which were filtered, washed with water and dried in vacuo overnight to give racemic 5-bromo-2- 15 aminotetraline-2-carboxylic acid (893 mg, 76% yield). LRMS (Electrospray): C₁₁H₁₂BrNO₂, calc. 269; observed: 270 (M+H), 272 (M+H), 268 (M-H), 270 (M-H).

Step 6:

20



A mixture of racemic 5-bromo-2-aminotetraline-2-carboxylic acid (882 mg, 3.27 mmole), triethylamine (0.60 ml, 4.30 mmole), 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu, 1.32 g, 3.91 mmole) in acetonitrile (30 ml) and water (30 ml) was stirred at room 25 temperature overnight. TLC analysis of the reaction the next day indicated the presence of starting material amino acid. 9-fluorenylmethyl succinimidyl carbonate (0.25 g), triethylamine (0.6 ml) and acetonitrile (5 ml) was added and the mixture was stirred at room temperature for another day. The reaction mixture was concentrated in vacuo to remove most of the acetonitrile, acidified to pH ~3 with 10% aqueous citric acid solution, and the white emulsion extracted twice

with methylene chloride. The combined organic layers were washed with water, brine, dried over magnesium sulfate. Filtration and concentration gave a crude oil which was purified by column chromatography (eluted with 2 → 5 → 10% methanol/methylene chloride) to give racemic Fmoc-5-bromo-2 aminotetraline-2-carboxylic acid (1.09 g, 68% yield) as a white solid.

5 HRMS (FAB): $C_{26}H_{22}BrNNaO_4$ ($M+Na$) calc. 514.0630; observed: 514.0643.

EXAMPLE 10

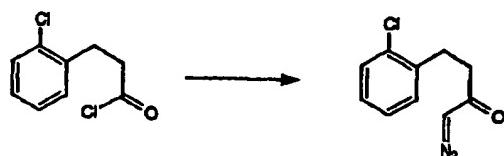
Preparation of Fmoc-(D,L)-5-chloro-2 aminotetraline-2-carboxylic acid (Fmoc-(D,L)-5-ClAtc-OH)

Step 1:



A mixture of 3-(2-chlorophenyl)propanoic acid (5.0 g, 27.1 mmole), thionyl chloride
15 (10.9 ml, 149 mmole) and toluene (75 ml) was refluxed for two hours. Concentration in vacuo
gave 3-(2-chlorophenyl)propanoyl chloride which was taken up in methylene chloride and used
in the next step without further purification.

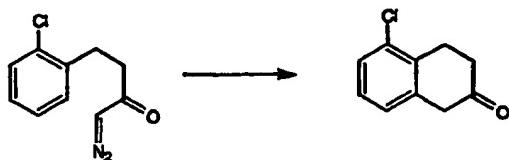
Step 2:



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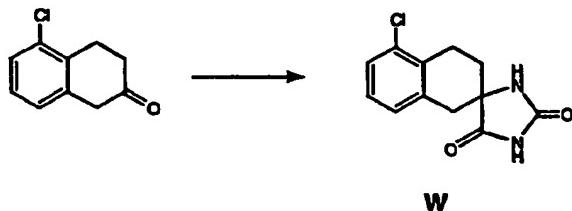
A solution of the above acid chloride (crude, 27.1 mmole) in methylene chloride was slowly added to a solution of diazomethane (generated from 17.8 g of 1-methyl-3-nitro-1-nitrosoguanidine) in ether (120 ml) cooled in an ice bath. The mixture was then warmed up to room temperature and stirred overnight. The mixture was concentrated in vacuo to give 1-diazo-25 4-(2-chlorophenyl)butan-2-one (5.87 g, > 100% over 2 steps) as a bright yellow oil. The compound was used in the next step without further purification. ¹H NMR (CDCl₃) δ 7.05-7.32 (4H, m, phenyl), 5.13 (1H, broad s, diazo), 3.00 (2H, t, benzylic), 2.57 (2H, m).

Step 3

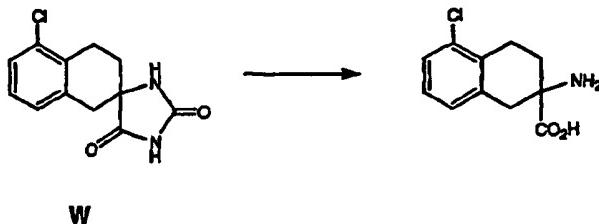


To a mixture of rhodium (II) acetate dimer (60 mg, 0.27 mmole) in methylene chloride (400 ml) under reflux was slowly added a solution of crude 1-diazo-4-(2-bromophenyl)butan-2-one (5.87 g, 27.1 mmole theoretical) in methylene chloride (50 ml). After the addition was complete, the mixture was refluxed for an extra twenty minutes. The mixture was cooled to room temperature, trifluoroacetic acid (6.0 ml) was added and the mixture was stirred at room temperature for two hours. The reaction was quenched with saturated sodium bicarbonate solution. The layers were separated and the methylene chloride layer was washed once more with saturated sodium bicarbonate solution. The combined aqueous layers were back-extracted with methylene chloride. The combined organic layers were dried over magnesium sulfate, filtered and concentrated in vacuo to give a brown oil. Purification by column chromatography (10 → 15% ethyl acetate/hexanes) gave 5-chloro-β-tetralone (3.32 g, 68% yield for steps 1 through 3) as a light brown oil. ^1H NMR (CDCl_3) δ 7.30 (1H, m, phenyl), 7.15 (1H, t, phenyl), 7.05 (1H, d, phenyl), 3.60 (2H, s, benzylic), 3.22 (2H, t, benzylic), 2.56 (2H, t).

Step 4:



A mixture of 5-chloro-β-tetralone (880 mg, 4.87 mmole), potassium cyanide (500 mg, 7.67 mmole), ammonium carbonate (2.85 g, 29.7 mmole), ethanol (24 ml) and water (6 ml) in a sealed, thick walled pressure flask was heated in a 80°C oil bath for 66 hours. After cooling to room temperature, the slurry was poured into ice-water and stirred at room temperature for a couple of hours. Filtration followed by air-drying gave hydantoin W (0.92 g, 75%) as a light beige solid. ^1H NMR (DMSO-d_6) δ 10.70 (1H, broad, NH), 8.25 (1H, broad s, NH), 7.0-7.3 (3H, m, phenyl). LRMS (Electrospray): $\text{C}_{12}\text{H}_{11}\text{ClN}_2\text{O}_2$, calc. 250; observed: 249 (M-H), 251 (M-H).

Step 5:

5 A mixture of hydantoin W (880 mg, 3.51 mmole), Ba(OH)₂·H₂O (3.40 g, 18.0 mmole) in water (50 ml, too dilute) in a sealed, thick walled pressure flask was heated in a 125°C oil bath for 2 days. The reaction mixture was cooled to room temperature, acidified to ~ pH 3 using 4N sulfuric acid while being stirred vigorously. The suspension was stirred in a boiling water bath for two hours and cooled to room temperature. The white suspension was filtered and the precipitates rinsed with water. The combined filtrate and washings were concentrated in vacuo to ~ 50 ml. Neutralization with concentrated ammonium hydroxide solution gave white precipitate which were filtered, washed with water and dried in vacuo overnight to give racemic 5-chloro-2-aminotetraline-2-carboxylic acid (788 mg, 99% yield). LRMS (Electrospray): C₁₁H₁₂ClNO₂, calc. 225; observed: 226 (M+H), 228 (M+H), 224 (M-H), 226 (M-H).

15

Step 6:

15 A mixture of racemic 5-chloro-2-aminotetraline-2-carboxylic acid (402 mg, 1.78 mmole), triethylamine (0.38 ml, 2.73 mmole), 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu, 904 mg, 2.68 mmole) in acetonitrile (20 ml) and water (20 ml) was stirred at room temperature for two days. TLC analysis of the reaction after two days indicated the presence of starting material amino acid. 9-fluorenylmethyl succinimidyl carbonate (0.12 g) and triethylamine (0.1 ml) was added and the mixture was stirred at room temperature for another day. The reaction mixture was concentrated in vacuo to remove most of the acetonitrile, acidified to pH ~3 with 10% aqueous citric acid solution, and the white emulsion extracted three times with ethyl acetate. The combined organic layers were washed with water, brine, dried over magnesium sulfate. Filtration and concentration gave a crude oil which was purified by column chromatography (eluted with 3 → 6 → 8% methanol/methylene chloride) to give racemic

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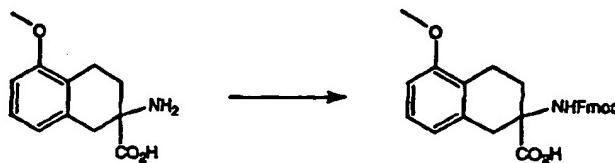
Fmoc-5-chloro-2-aminotetraline-2-carboxylic acid (540 mg, 68% yield) as a white solid. HRMS (EI): C₂₆H₂₂ClNO₄ (M) calc. 447.1237; observed: 447.1234.

5 **EXAMPLE 11**

Preparation of Fmoc-(D,L)-5-methoxy-2-aminotetraline-2-carboxylic acid (Fmoc-(D,L)-5-MeOAtc-OH)

Step 1:

10



A mixture of racemic 5-methoxy-2-aminotetraline-2-carboxylic acid (prepared according to Obrecht, D. *et. al. Helv. Chim Acta* 1992, 75, 1666) (802 mg, 3.62 mmole), triethylamine (0.62 ml, 4.45 mmole), 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu, 1.47 g, 4.36 mmole) in acetonitrile (25 ml) and water (25 ml) was stirred at room temperature for 30 hours.

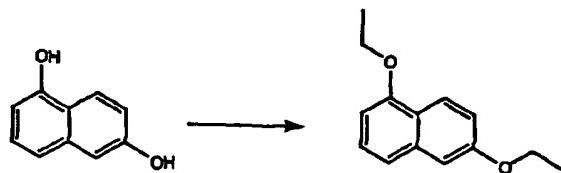
15 TLC analysis of the reaction indicated the presence of starting material amino acid. 9-fluorenylmethyl succinimidyl carbonate (370 mg) and triethylamine (0.6 ml) were added and the mixture was stirred at room temperature for another 24 hours. The reaction mixture was concentrated in vacuo to remove most of the acetonitrile, acidified to pH ~3 with 10% aqueous citric acid solution, and the white emulsion was extracted three times with ethyl acetate. The 20 combined organic layers were washed with water, brine and dried over magnesium sulfate. Filtration and concentration gave a crude oil which was purified by column chromatography (eluted with 1 → 3 → 5 → 10% methanol/methylene chloride) to give racemic Fmoc-5-methoxy-2-aminotetraline-2-carboxylic acid (1.14 g, 71% yield) as an off-white solid. HRMS (FAB): C₂₇H₂₆NO₅ (M+H) calc. 444.1812; observed: 444.1814.

25

EXAMPLE 12

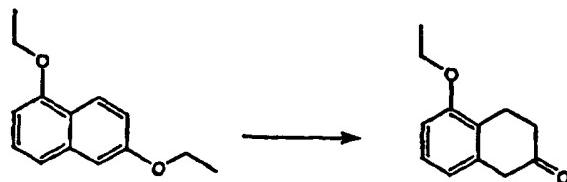
Preparation of Fmoc-(D,L)-5-ethoxy-2-aminotetraline-2-carboxylic acid (Fmoc-(D,L)-5-EtOAtc-OH)

5 Step 1:



A mixture of 1,6-dihydroxynaphthalene (5.02 g, 31.3 mmole), anhydrous potassium carbonate (52.0 g, 376 mmole), N,N-dimethylformamide (50 ml) and iodoethane (15 ml, 188 mmole) was stirred in a 35°C oil bath for 24 hours. The reaction mixture was filtered and the solid residue was rinsed thoroughly with ethyl ether. The filtrate and the washings were combined and concentrated in vacuo to remove most of the solvents. The brown residue was partitioned between water and ether and the layers were separated. The ether layer was washed with water. The combined aqueous layers were back extracted with ether. The ether extracts were combined, washed with brine and dried over magnesium sulfate. Filtration and concentration gave a crude brown solid (6.74 g, 99% yield). Recrystallization of the crude product from hot methanol gave 1,6-diethoxynaphthalene (4.36 g, 64% yield, first crop) as a light brown solid. ¹H NMR (CDCl₃) δ 8.20 (1H, d, phenyl), 7.06-7.36 (4H, m, phenyl), 6.66 (1H, dd, phenyl), 4.10-4.23 (4H, 2 sets of q, 2 CH₂), 1.45-1.56 (6H, 2 sets of t, 2 CH₃).

20 Step 2:



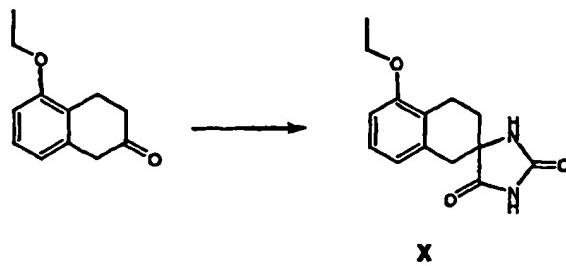
To a refluxing solution of 1,6-diethoxynaphthalene (4.15 g, 19.2 mmole) in absolute ethanol (100 ml) was carefully added small pieces of sodium metal (6.8 g, 296 mmole) over 60 minutes. The mixture was refluxed for another 90 minutes. TLC indicated the presence of unreacted starting material. Extra sodium metal (1.0 g, 43.5 mmole) was added and the reaction mixture was refluxed for another 60 minutes. The reaction was cooled to room temperature,

quenched with water and acidified with concentrated hydrochloric acid. The mixture was concentrated in vacuo to remove most of the ethanol. The aqueous mixture was extracted three times with ether. The combined organic layers were washed with water and dried over sodium sulfate. Filtration and concentration gave a brown solid which was dissolved in 1:1

5 ethanol/water (200 ml), then *p*-toluenesulfonic acid (400 mg) was added. The mixture was refluxed for 210 minutes. Extra *p*-toluenesulfonic acid (100 mg) was added and the mixture was refluxed for another 60 minutes. After cooling to room temperature, most of the ethanol was removed under reduced pressure. The aqueous mixture was extracted three times with ether and the combined organic layers were washed with water, saturated sodium chloride solution and

10 dried over sodium sulfate. Filtration and concentration gave a brwon oil which was purified by column chromatography (7% ethyl acetate/hexanes) to give 5-ethoxy- β -tetralone (2.43 g, 67% yield) as a light yellow oil. ^1H NMR (CDCl_3) δ 7.15 (1H, t, phenyl), 6.76 (1H, d, phenyl), 6.72 (1H, d, phenyl), 4.05 (2H, q, CH_2), 3.56 (2H, s, benzylic), 3.10 (2H, t, benzylic), 2.53 (2H, t), 1.44 (3H, t, CH_3).

15

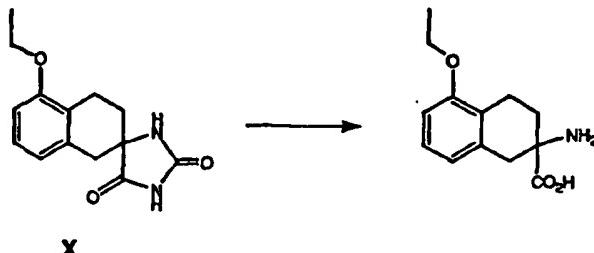
Step 3:

A mixture of 5-ethoxy- β -tetralone (2.23 g, 11.7 mmole), potassium cyanide (1.20 g, 18.4 mmole), ammonium carbonate (6.75 g, 70.2 mmole), ethanol (80 ml) and water (20 ml) in a

20 sealed, thick walled pressure flask was heated in a 80°C oil bath for 3 days. After cooling to room temperature, the slurry was poured into ice-water and stirred at room temperature for a couple of hours. Filtration followed by air-drying gave hydantoin X (2.69 g, 88%) as a beige solid. ^1H NMR (DMSO-d_6) δ 10.65 (1H, broad s, NH), 8.22 (1H, broad s, NH), 7.06 (1H, t, phenyl), 6.75 (1H, d, phenyl), 6.65 (1H, d, phenyl), 3.98 (2H, q, CH_2), 1.32 (3H, t, CH_3). LRMS

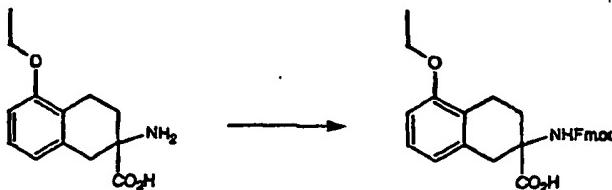
25 (Electrospray): $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_3$, calc. 259; observed: 258 (M-H).

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Step 4:

A mixture of hydantoin X (2.57 g, 9.87 mmole), Ba(OH)₂·H₂O (9.40 g, 49.6 mmole) in water (200 ml, too dilute) in a sealed, thick walled pressure flask was heated in a 105°C oil bath for 39 hours. Extra Ba(OH)₂·H₂O (9.40 g, 49.6 mmole) was added and the mixture was heated in a 125°C oil bath for an additional 21 hours. The reaction mixture was cooled to room temperature, acidified to ~ pH 3 using 4N sulfuric acid while being stirred vigorously. The suspension was stirred in a boiling water bath for one hour and cooled to room temperature. The white suspension was filtered and the precipitates rinsed with water. The combined filtrate and washings were concentrated in vacuo to ~ 75 ml. Neutralization with concentrated ammonium hydroxide solution gave white precipitate which were filtered, washed with water and air-dried to give racemic 5-ethoxy-2-aminotetraline-2-carboxylic acid (2.34 g, quantitative yield) as a light beige solid. LRMS (Electrospray): C₁₃H₁₇NO₃, calc. 235; observed: 236 (M+H), 234 (M-H).

15

Step 5:

A mixture of racemic 5-ethoxy-2-aminotetraline-2-carboxylic acid (2.22 g, 9.44 mmole), 20 triethylamine (2.00 ml, 14.3 mmole), 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu, 4.81 g, 14.3 mmole) in acetonitrile (75 ml) and water (75 ml) was stirred at room temperature for two days. TLC analysis of the reaction indicated the presence of starting material amino acid. 9-fluorenylmethyl succinimidyl carbonate (645 mg) and triethylamine (1.0 ml) was added and the mixture was stirred at room temperature for another day. The reaction mixture was 25 concentrated in vacuo to remove most of the acetonitrile, acidified to pH ~3 with 10% aqueous citric acid solution, and the white emulsion extracted three times with ethyl acetate. The combined organic layers were washed with water, brine, dried over magnesium sulfate.

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Filtration and concentration gave a crude oil which was purified by column chromatography (eluted with 3 → 5 → 10% methanol/methylene chloride) to give racemic Fmoc-5-ethoxy-2-aminotetraline-2-carboxylic acid (4.66 g, > quantitative yield) as a white solid. HRMS (FAB): C₂₈H₂₈NO₅ (M+H) calc. 458.1967; observed: 458.1985.

5

EXAMPLE 13

Preparation of Fmoc-(D,L)-5-isopropoxy-2-aminotetraline-2-carboxylic acid (Fmoc-(D,L) 5-iPrOAtc-OH)

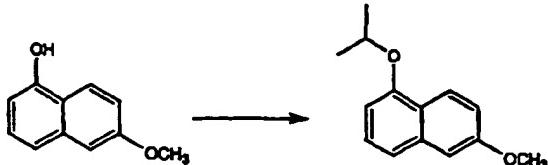
10 Step 1:



A mixture of 6-methoxy-1-tetralone (5.07 g, 28.8 mmole), 10% Pd/C (3.53 g, 3.32 mmole) in dry *p*-cymene (250 ml) was heated to reflux under argon for 38 hours. The reaction
15 mixture was cooled to room temperature, filtered over celite and the residue rinsed thoroughly with *p*-cymene. The filtrate and the washings were combined and extracted twice with 1N sodium hydroxide solution (2 x 70 ml). The combined aqueous extracts were acidified with 6N hydrochloric acid to pH ~3 and extracted three times with ether. The combined organic layers were washed with water, dried over anhydrous sodium sulfate. Filtration and concentration gave
20 crude 5-hydroxy-6-methoxynaphthalene (2.31 g, 46% yield) as a light brown solid which was used in the next step without further purification. LRMS (Electrospray): C₁₁H₁₀O₂, calc. 174; observed: 173 (M-H).

Step 2:

25



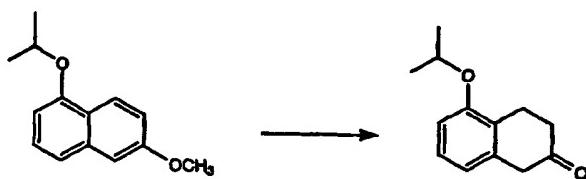
A mixture of 5-hydroxy-6-methoxynaphthalene (2.10 g, 12.1 mmole), cesium carbonate (19.7 g, 60.5 mmole), N,N-dimethylformamide (12 ml) and 2-bromopropane (3.50 ml, 36.9

- 95 -

mmole) was stirred in a 40°C oil bath overnight. The reaction mixture was filtered and the solid residue was rinsed thoroughly with ethyl ether. The filtrate and the washings were combined and concentrated in vacuo to remove most of the solvents. The brown residue was partitioned between water and ether and the layers were separated. The ether layer was washed with water.

- 5 The combined aqueous layers were back extracted with ether. The ether extracts were combined, washed with brine and dried over sodium sulfate. Filtration and concentration gave a crude which was purified by column chromatography (2.5 → 5% ethyl acetate/hexanes) to give 1-isopropoxy-6-methoxynaphthalene (2.23 g, 86% yield) as a light brown oil. ^1H NMR (CDCl_3) δ 8.17 (1H, d, phenyl), 7.05-7.38 (4H, m, phenyl), 6.72 (1H, dd, phenyl), 4.73 (1H, m, CH of iPr), 3.92 (3H, s, OCH_3), 1.42 (6H, d, 2 CH_3 of iPr).
- 10

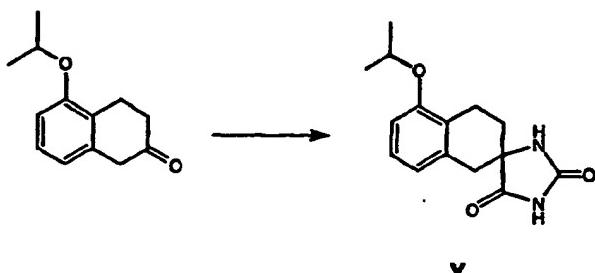
Step 3:



- To a refluxing solution of 1-isopropoxy-6-methoxynaphthalene (2.23 g, 10.3 mmole) in absolute ethanol (50 ml) was carefully added small pieces of sodium metal (3.6 g, 157 mmole) over 45 minutes. The mixture was refluxed for a further 120 minutes. The reaction was cooled to room temperature, quenched with water and acidified with concentrated hydrochloric acid. The mixture was concentrated in vacuo to remove most of the ethanol. The aqueous mixture was extracted three times with ether. The combined organic layers were washed with water and dried over sodium sulfate. Filtration and concentration gave a reddish oil which was dissolved in 1:1 ethanol/water (90 ml), then *p*-toluenesulfonic acid (200 mg) was added. The mixture was refluxed for 60 minutes. After cooling to room temperature, most of the ethanol was removed under reduced pressure. The aqueous mixture was extracted twice with ether and the combined organic layers were washed with water, saturated sodium chloride solution and dried over sodium sulfate. Filtration and concentration gave a reddish oil which was purified by column chromatography (8 → 15 % ethyl acetate/hexanes) to give 5-isopropoxy-β-tetralone (1.37 g, 65% yield) as a colorless oil. ^1H NMR (CDCl_3) δ 7.16 (1H, t, phenyl), 6.78 (1H, d, phenyl), 6.71 (1H, d, phenyl), 4.53 (1H, m, CH of iPr), 3.56 (2H, s, benzylic), 3.08 (2H, t, benzylic), 2.50 (2H, t), 1.37 (6H, d, 2 CH_3 of iPr).

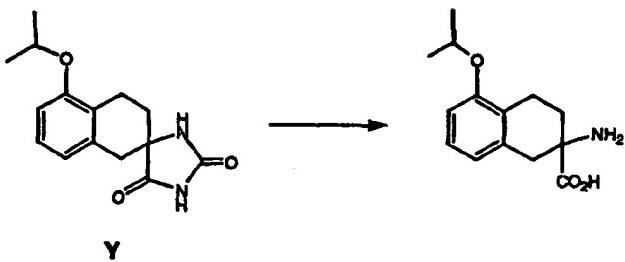
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Step 4:



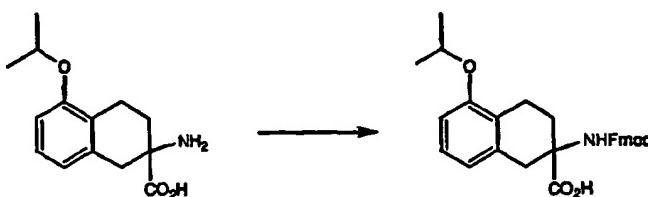
A mixture of 5-isopropoxy- β -tetralone (1.37 g, 6.71 mmole), potassium cyanide (660 mg, 10.1 mmole), ammonium carbonate (3.87 g, 40.3 mmole), ethanol (44 ml) and water (9 ml) 5 in a sealed, thick walled pressure flask was heated in a 80°C oil bath for 42 hours. After cooling to room temperature, the slurry was poured into ice-water and stirred at room temperature for a couple of hours. Filtration followed by air-drying gave hydantoin **Y** (1.64 g, 89%).

10 Step 5:



A mixture of hydantoin **Y** (1.64 g, 5.98 mmole), Ba(OH)₂·H₂O (5.66 g, 29.9 mmole) in water (25 ml) in a sealed, thick walled pressure flask was heated in a 100°C oil bath for 70 hours. The reaction mixture was cooled to room temperature, neutralized to ~ pH 7 using 4N 15 sulfuric acid while being stirred vigorously. The suspension was stirred in a boiling water bath for one hour and cooled to room temperature. Basified with 1N sodium hydroxide solution and the white suspension was filtered and the precipitates rinsed with water. The combined filtrate and washings were concentrated in vacuo to ~ 75 ml. Neutralization with concentrated hydrochloric acid solution gave white precipitate which were filtered, washed with water and 20 air-dried to give racemic 5-isopropoxy-2-aminotetraline-2-carboxylic acid (3.48 g, wet and containing inorganic salt, > quantitative yield). LRMS (Electrospray): C₁₄H₁₉NO₃, calc. 249; observed: 248 (M-H).

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Step 6:

A mixture of racemic 5-isopropoxy-2-aminotetraline-2-carboxylic acid (3.48 g, 5.98 mmole theoretical), triethylamine (1.10 ml, 7.89 mmole), 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu, 2.62 g, 7.77 mmole) in acetonitrile (30 ml) and water (30 ml) was stirred at room temperature for one day. TLC analysis of the reaction indicated the presence of starting material amino acid. 9-fluorenylmethyl succinimidyl carbonate (500 mg) was added and the mixture was stirred at room temperature for another day. The reaction mixture was concentrated in vacuo to remove most of the acetonitrile, acidified to pH ~3 with 10% aqueous citric acid solution, and the white emulsion extracted three times with methylene chloride. The combined organic layers were washed with water, brine, dried over magnesium sulfate. Filtration and concentration gave a crude oil which was purified by column chromatography (eluted with 1 → 2 → 5 → 8% methanol/methylene chloride) to give racemic Fmoc-5-isopropoxy-2-aminotetraline-2-carboxylic acid (0.50 g, 18% yield over 2 steps) as a white solid. HRMS (FAB): C₂₉H₃₀NO₅ (M+H) calc. 472.2124; observed: 472.2117.

EXAMPLE 14

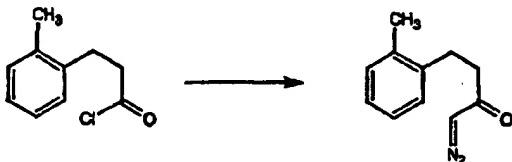
Preparation of Fmoc-(D,L)-5-methyl-2-aminotetraline-2-carboxylic acid (Fmoc-(D,L)-5-MeAtc-OH)

Step 1:

A mixture of 2-methylhydrocinnamic acid (3.0 g, 18.3 mmole), oxalyl chloride (3.19 ml, 36.6 mmole) and methylene chloride (30 ml) was cooled in an ice bath and N,N-dimethylformamide (0.14 ml, 1.81 mmole) was added dropwise. The mixture was stirred at room temperature overnight. Concentration in vacuo gave 3-(2-methylphenyl)propanoyl chloride which was taken up in methylene chloride and used in the next step as a crude.

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Step 2:



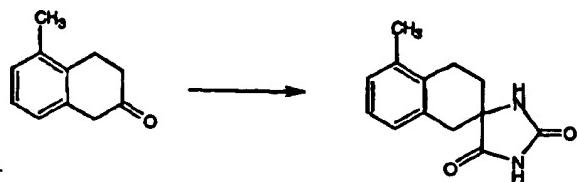
A solution of the above acid chloride (crude, 18.3 mmole) in methylene chloride was slowly added to a solution of diazomethane (generated from 11.9 g of 1-methyl-3-nitro-1-nitrosoguanidine) in ether (80 ml) cooled in an ice bath. The mixture was then warmed up to room temperature and stirred overnight. The mixture was concentrated in vacuo and purified by column chromatography (10 → 20% ethyl acetate/hexanes) to give 1-diazo-4-(2-methylphenyl)butan-2-one (2.08 g, 60% over 2 steps) as a bright yellow oil.

10 Step 3:



To a mixture of rhodium (II) acetate dimer (24 mg, 0.109 mmole) in methylene chloride (200 ml) under reflux was slowly added a solution of 1-diazo-4-(2-methylphenyl)butan-2-one (2.08 g, 11.1 mmole) in methylene chloride (50 ml) over 180 minutes. After the addition was complete, the mixture was refluxed for an extra twenty minutes. The mixture was cooled to room temperature, trifluoroacetic acid (2.40 ml) was added and the mixture was stirred at room temperature for an hour. The reaction was quenched with saturated sodium bicarbonate solution. The layers were separated and the methylene chloride layer was washed once more with saturated sodium bicarbonate solution. The combined aqueous layers were back-extracted with methylene chloride. The combined organic layers were dried over magnesium sulfate, filtered and concentrated in vacuo to give a crude brown oil. Purification by column chromatography (15% ethyl acetate/hexanes) gave 5-methyl-β-tetralone (1.48 g, 84% yield) as a light brown oil.
¹H NMR (CDCl₃) δ 6.90-7.20 (3H, m, phenyl), 3.58 (2H, s, benzylic), 3.03 (2H, t, benzylic), 2.55 (2H, t), 2.34 (3H, s, CH₃).

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Step 4:

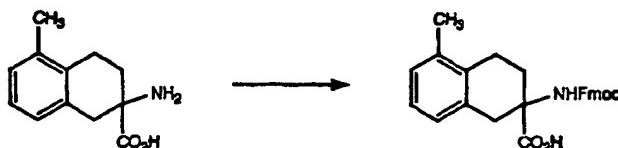
A mixture of 5-methyl- β -tetralone (1.48 g, 9.24 mmole), potassium cyanide (902 mg, 13.9 mmole), ammonium carbonate (5.33 g, 55.5 mmole), ethanol (45 ml) and water (9 ml) in a 5 sealed, thick walled pressure flask was heated in a 80°C oil bath for 3 days. After cooling to room temperature, the slurry was poured into ice-water and stirred at room temperature for a couple of hours. Filtration followed by air-drying gave crude hydantoin Z (1.81 g, 85% yield) as a beige solid. ^1H NMR (DMSO- d_6) δ 10.66 (1H, broad s, NH), 8.22 (1H, broad s, NH), 6.85-7.05 (3H, m, phenyl), 2.17 (3H, s, CH_3).

10

Step 5:

A mixture of hydantoin Z (1.80 g, 7.82 mmole), $\text{Ba}(\text{OH})_2 \cdot \text{H}_2\text{O}$ (7.40 g, 39.1 mmole) in water (28 ml) in a sealed, thick walled pressure flask was heated in a 125°C oil bath for 88 15 hours. The reaction mixture was cooled to room temperature, acidified to ~ pH 3 using 4N sulfuric acid while being stirred vigorously. The suspension was stirred in a boiling water bath for an hour and cooled to room temperature. The white suspension was filtered and the precipitates rinsed with water. The combined filtrate and washings were concentrated in vacuo to ~ 50 ml. Neutralization with concentrated ammonium hydroxide solution gave white 20 precipitate which were filtered, washed with water and air-dried to give racemic 5-methyl-2-aminotetraline-2-carboxylic acid (1.05 g, 65% yield) as a beige solid. LRMS (Electrospray): $\text{C}_{12}\text{H}_{15}\text{NO}_2$, calc. 205; observed: 206 ($\text{M}+\text{H}$).

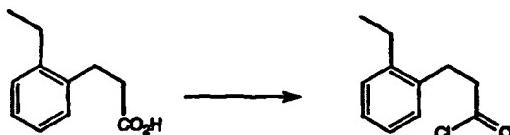
- 100 -

Step 6:

5 A mixture of racemic 5-methyl-2-aminotetraline-2-carboxylic acid (1.05 g, 5.12 mmole), triethylamine (0.93 ml, 6.67 mmole), 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu, 2.24 g, 6.64 mmole) in acetonitrile (30 ml) and water (30 ml) was stirred at room temperature for 2 days. TLC analysis of the reaction indicated the presence of starting material amino acid. 9-fluorenylmethyl succinimidyl carbonate (520 mg) was added and the mixture was stirred at 10 room temperature for another 24 hours. The reaction mixture was concentrated in vacuo to remove most of the acetonitrile, acidified to pH ~3 with 10% aqueous citric acid solution, and the white emulsion was extracted twice with methylene chloride. The combined organic layers were washed with water, brine and dried over magnesium sulfate. Filtration and concentration gave a crude oil which was purified by column chromatography (eluted with 2 → 5 → 8% 15 methanol/methylene chloride) to give racemic Fmoc-5-methyl-2-aminotetraline-2-carboxylic acid (1.62 g, 74% yield) as an light brown solid. HRMS (FAB): C₂₇H₂₆NO₄ (M+H) calc. 428.1862; observed: 428.1844.

20 EXAMPLE 15

Preparation of Fmoc-(D,L)-5-ethyl-2 aminotetraline-2-carboxylic acid (Fmoc-(D,L) 5-EtAtc-OH)

Step 1:

25 A mixture of 3-(2-ethylphenyl)propanoic acid (prepared in 3 steps from 1-ethyl-2-iodobenzene, 4.24 g, 23.8 mmole), thionyl chloride (9.50 ml, 130 mmole) and toluene (100 ml) was refluxed for 2 hours. Concentration in vacuo gave 3-(2-ethylphenyl)propanoyl chloride which was taken up in methylene chloride and used in the next step as a crude.

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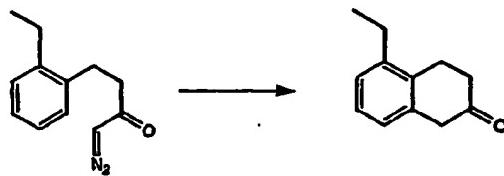
Step 2:



A solution of 3-(2-ethylphenyl)propanoyl chloride (crude, 23.8 mmole) in methylene chloride was slowly added to a solution of diazomethane (generated from 15.6 g of 1-methyl-3-nitro-1-nitrosoguanidine) in ether (100 ml) cooled in an ice bath. The mixture was then warmed up to room temperature and stirred overnight. The mixture was concentrated in vacuo and purified by column chromatography (10 → 20% ethyl acetate/hexanes) to give 1-diazo-4-(2-ethylphenyl)butan-2-one (3.47 g, 72% over 2 steps). ^1H NMR (CDCl_3) δ 7.1-7.25 (4H, m, phenyl), 5.21 (1H, broad s, diazo), 2.97 (2H, m, CH_2 of ethyl), 1.20 (3H, t, CH_3).

10

Step 3:



To a mixture of rhodium (II) acetate dimer (38 mg, 0.172 mmole) in methylene chloride (300 ml) under reflux was slowly added a solution of 1-diazo-4-(2-ethylphenyl)butan-2-one (3.47 g, 17.2 mmole) in methylene chloride (50 ml) over 90 minutes. After the addition was complete, the mixture was refluxed for an extra twenty minutes. The mixture was cooled to room temperature, trifluoroacetic acid (3.75 ml) was added and the mixture was stirred at room temperature for an hour. The reaction was quenched with saturated sodium bicarbonate solution.

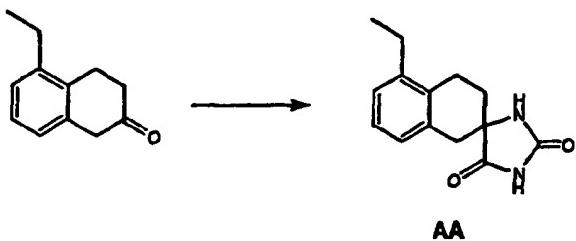
15 The layers were separated and the methylene chloride layer was washed once more with saturated sodium bicarbonate solution. The combined aqueous layers were back-extracted with methylene chloride. The combined organic layers were dried over magnesium sulfate, filtered and concentrated in vacuo to give crude 5-ethyl- β -tetralone (3.09 g, > quantitative yield) as a reddish-brown oil. The compound was used in the next step without further purification. ^1H

20 NMR (CDCl_3) δ 6.9-7.2 (3H, m, phenyl), 3.58 (2H, s, benzylic), 3.08 (2H, s, benzylic), 2.70 (2H, q, CH_2 of ethyl), 2.52 (2H, t, benzylic), 1.20 (3H, t, CH_3 of ethyl).

25

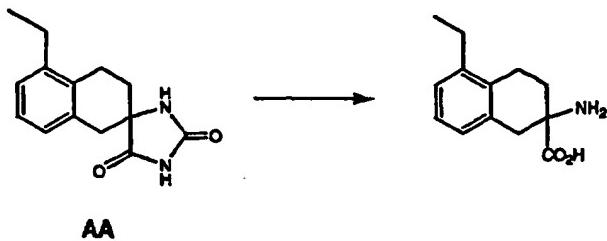
- 102 -

Step 4:

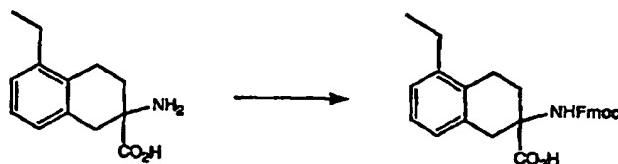


A mixture of 5-ethyl- β -tetralone (3.09 g, 17.7 mmole), potassium cyanide (1.73 g, 26.6 mmole), ammonium carbonate (10.2 g, 106 mmole), ethanol (80 ml) and water (16 ml) in a sealed, thick walled pressure flask was heated in a 80°C oil bath for 48 hours. After cooling to room temperature, the white slurry was poured into ice-water and stirred at room temperature for a couple of hours. Filtration followed by air-drying gave hydantoin AA (3.85 g, 92% yield over 2 steps) as a light beige solid. ^1H NMR (DMSO- d_6) δ 10.67 (1H, broad s, NH), 8.26 (1H, broad s, NH), 6.8-7.1 (3H, m, phenyl), 1.13 (3H, t, CH_3). LRMS (Electrospray): $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_2$, calc. 244; observed: 243 ($\text{M}-\text{H}$).

Step 5:



A mixture of hydantoin AA (1.00 g, 4.09 mmole), $\text{Ba}(\text{OH})_2 \cdot \text{H}_2\text{O}$ (4.00 g, 21.1 mmole) in water (20 ml) in a sealed, thick walled pressure flask was heated in a 125°C oil bath for 48 hours. The reaction mixture was cooled to room temperature, acidified to ~ pH 3 using 4N sulfuric acid while being stirred vigorously. The suspension was stirred in a boiling water bath for two hours and cooled to room temperature. The white suspension was filtered and the precipitates rinsed with water. The combined filtrate and washings were concentrated in vacuo to ~ 50 ml. Neutralization with concentrated ammonium hydroxide solution gave white precipitate which were filtered, washed with water and dried in vacuo overnight to give racemic 5-ethyl-2-aminotetraline-2-carboxylic acid (796 mg, 89% yield). LRMS (Electrospray): $\text{C}_{13}\text{H}_{17}\text{NO}_2$, calc. 219; observed: 220 ($\text{M}+\text{H}$).

Step 6:

5 A mixture of racemic 5-ethyl-2-aminotetraline-2-carboxylic acid (765 mg, 3.49 mmole), triethylamine (1.0 ml, 7.17 mmole), 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu, 1.79 g, 5.31 mmole) in acetonitrile (40 ml) and water (40 ml) was stirred at room temperature for 2 days. The reaction mixture was concentrated in vacuo to remove most of the acetonitrile, acidified to pH ~3 with 10% aqueous citric acid solution, and the white emulsion extracted twice
10 with methylene chloride, twice with ethyl acetate. The methylene chloride extracts were washed with water, brine and dried over magnesium sulfate. The ethyl acetate extracts were washed with water, brine and dried over magnesium sulfate. Filtration and concentration gave a crude oil which was purified by column chromatography (eluted with 2 → 5 → 8%
15 methanol/methylene chloride) to give racemic Fmoc-5-ethyl-2-aminotetraline-2-carboxylic acid (330 mg, 21% yield) as a white solid. HRMS (FAB): C₂₈H₂₈NO₄ (M+H) calc. 442.2018; observed: 442.2010.

20 EXAMPLE 16

Preparation of Fmoc-(D,L)-5-isopropyl-2-aminotetraline-2-carboxylic acid (Fmoc-(D,L)-5-iPrAtc-OH)

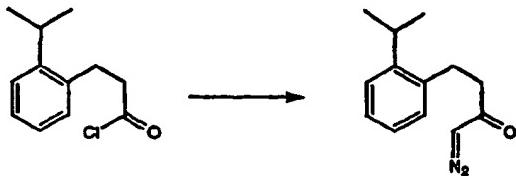
25 Step 1:

A mixture of 3-(2-isopropylphenyl)propanoic acid (prepared in 3 steps from 1-isopropyl-2-iodobenzene, 2.01 g, 10.5 mmole), thionyl chloride (4.30 ml, 59.0 mmole) and toluene (40 ml)

was refluxed for 2 hours. Concentration in vacuo gave 3-(2-isopropylphenyl)propanoyl chloride which was taken up in methylene chloride and used in the next step as a crude.

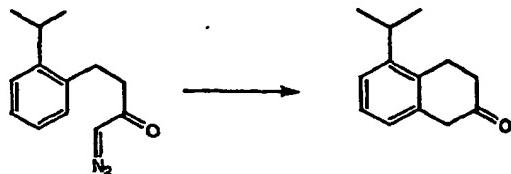
Step 2:

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- A solution of 3-(2-isopropylphenyl)propanoyl chloride (crude, 10.5 mmole) in methylene chloride was slowly added to a solution of diazomethane (generated from 6.95 g of 1-methyl-3-nitro-1-nitrosoguanidine) in ether (50 ml) cooled in an ice bath. The mixture was then warmed up to room temperature and stirred overnight. The mixture was concentrated in vacuo and purified by column chromatography (20% ethyl acetate/hexanes) to give 1-diazo-4-(2-isopropylphenyl)butan-2-one (1.87 g, 82% over 2 steps) as a bright yellow oil. ¹H NMR (CDCl₃) δ 7.10-7.30 (4H, m, phenyl), 5.21 (1H, broad s, diazo), 3.15 (1H, m, CH of iPr), 3.00 (2H, t, benzylic), 2.57 (2H, m), 1.24 (6H, d, 2 CH₃ of iPr).

15 **Step 3:**

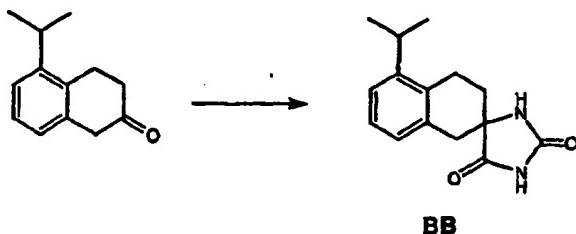


- To a mixture of rhodium (II) acetate dimer (20 mg, 0.091 mmole) in methylene chloride (160 ml) under reflux was slowly added a solution of 1-diazo-4-(2-bromophenyl)butan-2-one (1.87 g, 8.65 mmole) in methylene chloride (25 ml) over 60 minutes. After the addition was complete, the mixture was refluxed for an extra fifteen minutes. The mixture was cooled to room temperature, trifluoroacetic acid (1.90 ml) was added and the mixture was stirred at room temperature for 45 minutes. The reaction was quenched with saturated sodium bicarbonate solution. The layers were separated and the methylene chloride layer was washed once more with saturated sodium bicarbonate solution. The combined aqueous layers were back-extracted with methylene chloride. The combined organic layers were dried over magnesium sulfate, filtered and concentrated in vacuo to give a crude brown oil. Purification by column

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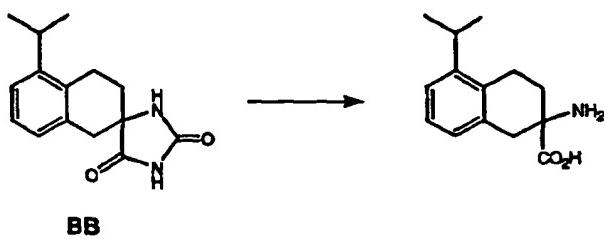
chromatography (5% ethyl acetate/hexanes) gave 5-isopropyl- β -tetralone (1.57 g, 96% yield) as a light yellow oil. ^1H NMR (CDCl_3) δ 6.93-7.22 (3H, m, phenyl), 3.59 (2H, s, benzylic), 3.24 (1H, m, CH of iPr), 3.12 (2H, t, benzylic), 2.52 (2H, t), 1.27 (6H, d, 2 CH₃ of iPr).

5 Step 4:



A mixture of 5-isopropyl- β -tetralone (1.57 g, 8.34 mmole), potassium cyanide (0.82 g, 12.6 mmole), ammonium carbonate (4.81 g, 50.1 mmole), ethanol (40 ml) and water (10 ml) in a sealed, thick walled pressure flask was heated in a 80°C oil bath for 48 hours. After cooling to room temperature, the brown slurry was poured into ice-water and stirred at room temperature for a couple of hours. Filtration followed by air-drying gave crude hydantoin BB as a beige solid which was used in the next step without further purification. ^1H NMR (DMSO-d_6) δ 10.69 (1H, broad s, NH), 8.30 (1H, broad s, NH), 6.85-7.32 (3H, m, phenyl), 1.15 (6H, t, CH₃). LRMS (Electrospray): C₁₅H₁₈N₂O₂, calc. 258; observed: 539 (2M+Na).

Step 5:

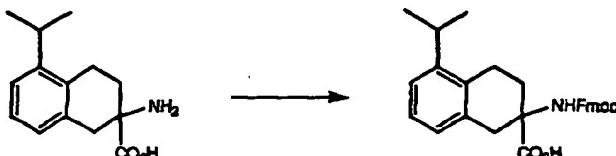


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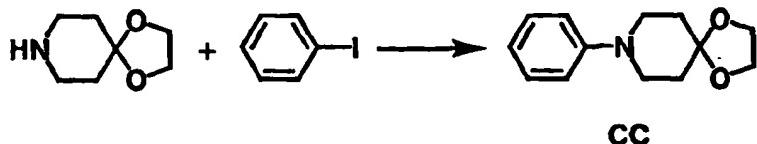
A mixture of hydantoin BB (crude, 8.34 mmole theoretical), Ba(OH)₂. H₂O (7.90 g, 41.7 mmole) in water (40 ml) in a sealed, thick walled pressure flask was heated in a 125°C oil bath for 38 hours. The reaction mixture was cooled to room temperature, acidified to ~ pH 3 using 4N sulfuric acid while being stirred vigorously. The suspension was stirred in a boiling water bath for two hours and cooled to room temperature. The white suspension was filtered and the precipitates rinsed with water. The combined filtrate and washings were concentrated in vacuo

to ~ 50 ml. Neutralization with concentrated ammonium hydroxide solution gave white precipitate which were filtered, washed with water and dried in vacuo overnight to give racemic 5-isopropyl-2-aminotetraline-2-carboxylic acid (1.23 g, 63% yield over 2 steps) as a beige solid.
LRMS (Electrospray): C₁₄H₁₉NO₂, calc. 233; observed: 232 (M-H).

5

Step 6:

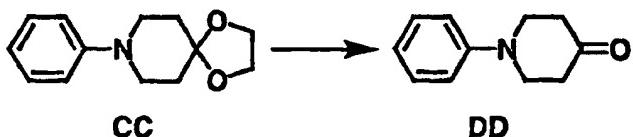
A mixture of racemic 5-isopropyl-2-aminotetraline-2-carboxylic acid (250 mg, 1.07 mmole), triethylamine (1.2 ml, 8.61 mmole), 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu, 2.70 g, 8.00 mmole) in acetonitrile (30 ml) and water (30 ml) was stirred at room temperature for 2 days. The reaction mixture was concentrated in vacuo to remove most of the acetonitrile, acidified to pH ~3 with 10% aqueous citric acid solution, and the white emulsion was extracted with ethyl acetate. The organic layer was washed with water, brine and dried over sodium sulfate. Filtration and concentration gave a crude oil which was purified by column chromatography (eluted with 2 → 5 → 8% methanol/methylene chloride) to give racemic Fmoc-5-isopropyl-2-aminotetraline-2-carboxylic acid (208 mg, 43% yield) as an off-white foam.
HRMS (FAB): C₂₉H₃₀NO₄ (M+H) calc. 456.2175; observed: 456.2184.

20 EXAMPLE 17**Preparation of Fmoc-4-amino-1-phenylpiperidine-4-carboxylic acid (Fmoc-Appc-OH)****Step 1:**

To a solution of iodobenzene (6.37 g, 3.5 mL, 31.2 mmole), 1,4-dioxa-8-azaspiro [4.5] decane (10.32 g, 9.3 mL, 72.2 mmole, 2.3 equiv) and sodium tert-butoxide (8.0 g, 83.3 mmole, 2.7 equiv) in dry dioxane (120 mL) were added tris(dibenzylideneacetone)dipalladium(0) (91 mg, 0.1 mmol) and tri-*o*-tolylphosphine (180 mg, 0.591 mmol). The reaction was heated at 90 °C for 26 hrs. The resulting reaction mixture was concentrated to remove solvent. The residue

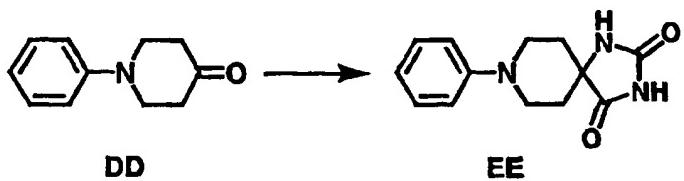
was treated with water and extracted with EtOAc. The combined organic extracts were combined, washed with brine, dried over Na₂SO₄ and concentrated to give a brown oil. This crude product was purified on flash chromatography (hexane/EtOAc, 95/5 to 75/25) to provide the pure product CC as a slightly yellow solid (6.08 g, 89%). ¹H NMR (CDCl₃), 7.25 (ddt, 2H), 5 6.95 (dd, 2H), 6.84 (t, 1H), 4.00 (s, 4H), 3.32 (t, 4H) and 1.84 (t, 4H); MS (electrospray) m/e 220 (M+H), Calcd for C₁₃H₁₇NO₂, 219.

Step 2:



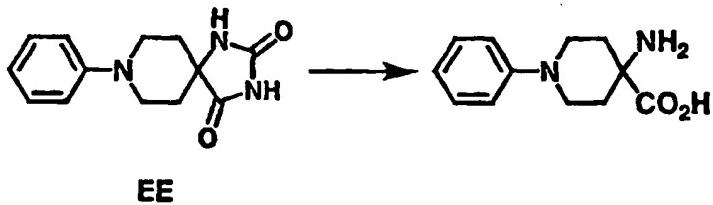
To a solution of the ketal CC (3.22 g, 15.16 mmol) in acetone (100 mL) was added 6N hydrochloric acid (50 mL) and the reaction was heated at reflux overnight. The resulting reaction mixture was concentrated to remove solvent. The residue was taken up in EtOAc and neutralized with aqueous 6N NaOH solution. The layers were separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄ and concentrated. The crude product was purified on flash chromatography (hexane/EtOAc, 80/20→60/40) to give the product DD as a yellow oil (2.58 g, 97%). MS (electrospray) m/e 176 (M+H), Calcd for C₁₁H₁₃NO, 175.

Step 3:



To a solution of the ketone DD (2.53 g, 14.46 mmol) in ethanol (75 mL) and water (25 mL) in a glass pressure bottle, were added ammonium carbonate (12.9 g, 134.3 mmole, 9 equiv.) and potassium cyanide (2.11 g, 32.5 mmol, 2 equiv.). The mixture was heated at 80-90 °C for 18 hrs. The cooled reaction mixture was concentrated in vacuo and the residue was treated with water, extracted with EtOAc (4x). The combined organic extracts were washed with water, dried over anhydrous Na₂SO₄ and concentrated to give the spectroscopically pure hydantoin EE as a white solid (3.36 g, 95% yield). MS (electrospray) m/e 246 (M+H), Calcd for C₁₃H₁₅N₃O₂, 245.

Step 4:



The hydantoin EE (3.36 g) was suspended in aqueous NaOH (6N, 100 mL) and heated at 5 130 °C for 2-3 days. Upon completion (by HPLC) of the hydrolysis, the reaction mixture was neutralized with conc. HCl to slightly acidic (pH ~6). The resulting slurry was filtered, washed with water and dried to give 4-amino-1-phenylpiperidine-4-carboxylic acid (APPC) as a white solid (5.26 g, >100 % yield, wet and contaminated with inorganic salt), which showed a single peak on HPLC and used directly for the next step. MS (electrospray) m/e 221 (M+H), Calcd for 10 $C_{12}H_{16}N_2O_2$, 220.

Step 5:



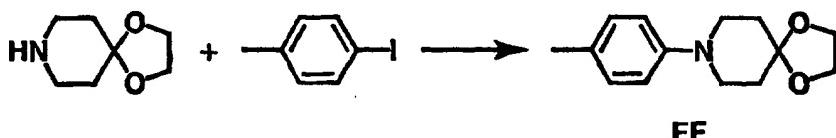
The crude 4-amino-1-phenylpiperidine-4-carboxylic acid (APPC) from the last step was 15 suspended in dioxane (80 mL) and aqueous 10% Na_2CO_3 (40 mL), treated with Fmoc-Cl (5.3 g, 20.57 mmole, 1.5 equiv) and was stirred vigorously overnight. The reaction mixture was then concentrated to remove dioxane, neutralized with 6N HCl to slightly acidic (pH 6) and extracted with EtOAc. The combined organic extracts were washed with brine and dried over Na_2SO_4 . Removal of the solvent gave the crude product which was purified on flash chromatography 20 (hexane/EtOAc to $CH_2Cl_2/MeOH$) to give pure Fmoc-APPC (4.91 g, 81% overall yield for two steps). 1H NMR(DMSO- d_6), 7.88 (d, 2H), 7.74 (d, 2H), 7.19-7.42 (m, 8H), 4.20-4.31 (m, 3H); HRMS m/z 465.1788, Calcd for $C_{27}H_{26}N_2O_4Na$, 465.1791

EXAMPLE 18

Preparation of Fmoc-4-amino-1-(4-methylphenyl)piperidine-4-carboxylic acid (Fmoc-4-MeAppc-OH)

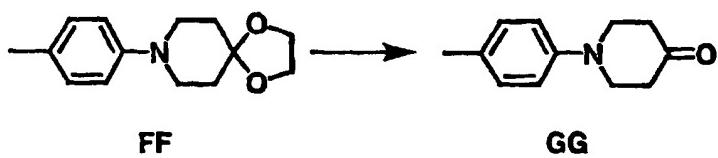
Step 1:

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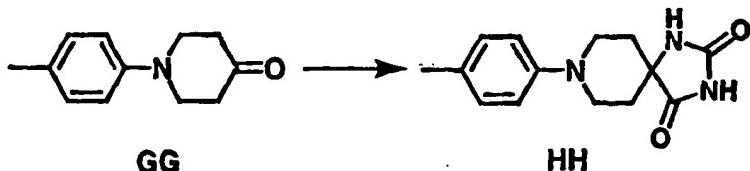
To a solution of 4-iodotoluene (2.12 g, 9.7 mmol), 1,4-dioxa-8-azaspiro[4.5]decane (2.8 mL, 3.12 g, 21.82 mmol, 2.2 equiv) and sodium tert-butoxide (2.6 g, 27.08 mmol, 2.8 equiv) in dry dioxane (40 mL) were added tris(dibenzylideneacetone)dipalladium (0) (44.4 mg, 0.0485 mmol) and tri-*o*-tolylphosphine (59.0 mg, 0.194 mmol). The reaction was heated at 90 °C for 26 hrs. The resulting reaction mixture was concentrated to remove solvent. The residue was treated with water and extracted with EtOAc. The combined organic extracts were combined, washed with brine, dried over Na₂SO₄ and concentrated to give brown oil. This crude product was purified on flash chromatography (hexane/EtOAc, 95/5 to 75/25) to provide the pure product FF as a slightly yellow solid (1.937 g, 85%). ¹H NMR (CDCl₃), 7.06 (d, 2H), 6.87 (d, 2H), 3.99 (s, 4H), 3.26 (t, 4H), 2.26 (s, 3H) and 1.85 (t, 4H).

Step 2:



To a solution of the ketal FF (1.58 g, 6.79 mmol) in acetone (50 mL) was added 6N hydrochloric acid (25 mL) and the reaction was heated at reflux overnight. The resulting reaction mixture was concentrated to remove solvent. The residue was taken up in EtOAc and neutralized with aqueous 6N NaOH solution. The layers were separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄ and concentrated. The crude product was purified on flash chromatography (hexane/EtOAc, 90/10 → 70/30) to give the product GG as a yellow oil (1.27 g, 98%). MS (electrospray) *m/e* 190 (M+H), Calcd for C₁₂H₁₅NO, 189.

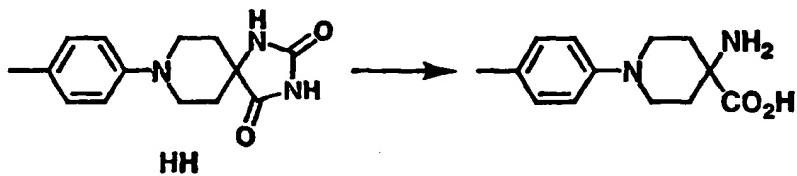
Step 3:



To a solution of the ketone GG (1.17 g, 6.18 mmol) in ethanol (60 mL) and water (20 mL) in a glass pressure bottle, were added ammonium carbonate (4.74 g, 49.44 mmole, 8 equiv.) and potassium cyanide (1.01 g, 15.54 mmol, 2.5 equiv.). The mixture was heated at 90 °C for 22 hrs. The cooled reaction mixture was concentrated in vacuo and the residue was treated with water, extracted with EtOAc (4x). The combined organic extracts were washed with water, dried over anhydrous Na₂SO₄ and concentrated to give the spectroscopically pure hydantoin HH as a white solid (1.554 g, 97% yield). MS (electrospray) *m/e* 260 (M+H), Calcd for C₁₄H₁₇N₃O₂, 259.

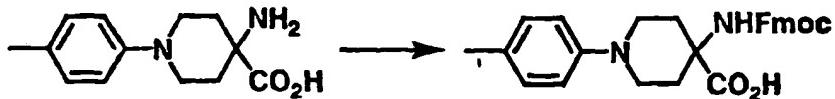
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Step 4:



The hydantoin HH (1.502 g) was suspended in aqueous NaOH (6N, 40 mL) and heated at 130 °C for 4 days. Upon completion (by HPLC) of the hydrolysis, the reaction mixture was neutralized with conc. HCl to slightly acidic (pH ~6). The resulting slurry was filtered, washed with water and dried to give 4-amino-1-(4-methylphenyl)piperidine-4-carboxylic acid (4-MeAPPC) as a white solid (2.10 g, >100 % yield, wet and contaminated with inorganic salt), which showed a single peak on HPLC and used directly in the next step. MS (electrospray) *m/e* 235 (M+H), Calcd for C₁₃H₁₈N₂O₂, 234.

20 Step 5:



The crude 4-amino-1-(4-methylphenyl)piperidine-4-carboxylic acid (4-MeAPPC) from the last step was suspended in dioxane (80 mL) and aqueous 10% Na₂CO₃ (40 ml), treated with

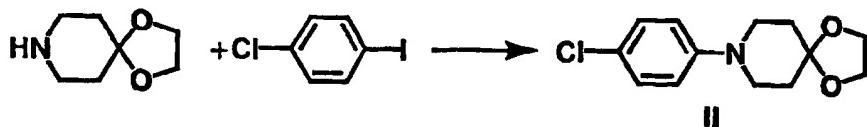
- 111 -

Fmoc-Cl (2.2 g, 8.59 mmole, 1.5 equiv) and was stirred vigorously overnight. The reaction mixture was then concentrated to remove dioxane, neutralized with 6N HCl to slightly acidic (pH 6) and extracted with EtOAc. The combined organic extracts were washed with brine and dried over Na₂SO₄. Removal of the solvent gave the crude product which was purified on flash chromatography (hexane/EtOAc to CH₂Cl₂/MeOH) to give pure Fmoc-4-MeAPPC (2.16 g, 82% overall yield for two steps). ¹H NMR (DMSO-d₆): 7.88 (d, 2H), 7.72 (d, 2H), 7.39 (t, 2H), 7.30 (td, 2H), 6.99 (d, 2H), 6.82 (d, 2H), 2.18 (s, 3H); MS (electrospray) m/e 457 (M+H), Calcd for C₂₈H₂₈N₂O₄, 456.

10 **EXAMPLE 19**

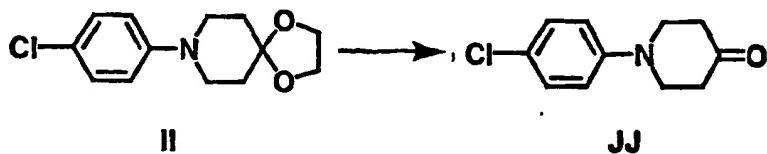
Preparation of Fmoc-4-amino-1-(4-chlorophenyl)piperidine-4-carboxylic acid (Fmoc-4-ClAppc-OH)

Step 1:



15 To a solution of 1-chloro-4-iodobenzene (2.38 g, 10.0 mmole), 1,4-dioxa-8-azaspiro [4.5] decane (3.1 mL, 3.44 g, 24.0 mmole, 2.4 equiv) and sodium tert-butoxide (2.68 g, 28.0 mmole, 2.8 equiv) in dry dioxane (40 mL) were added tris(dibenzylideneacetone)dipalladium(0) (45.5 mg, 0.0497 mmol) and tri-*o*-tolyl-phosphine (61 mg, 0.20 mmol). The reaction was heated at 90 °C for 9 hrs. The resulting reaction mixture was concentrated to remove solvent. The residue
20 was treated with water and extracted with EtOAc. The combined organic extracts were combined, washed with brine, dried over Na₂SO₄ and concentrated to give a brown oil. This crude product was purified on flash chromatography (hexane/EtOAc, 95/5 to 75/25) to provide the pure product II as a slightly yellow solid (2.17 g, 86%). ¹H NMR(CDCl₃), 7.18 (dt, 2H), 6.85 (dt, 2H), 3.98 (s, 4H), 3.28 (t, 4H) and 1.82 (t, 4H).

25 Step 2:

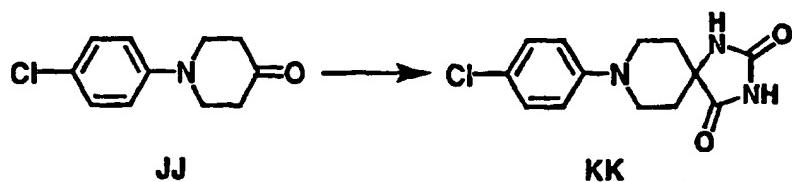


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To a solution of the ketal II (2.123 g, 8.39 mmole) in acetone (75 mL) was added 6N hydrochloric acid (30 mL) and the reaction was heated at reflux overnight. The resulting reaction mixture was concentrated to remove solvent. The residue was taken up in EtOAc and neutralized with aqueous 6N NaOH solution. The layers were separated and the aqueous layer 5 was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄ and concentrated. The crude product was purified on flash chromatography (hexane/EtOAc, 95/5 → 70/30) to give the product JJ as a yellow solid (1.515 g, 86%). MS (electrospray) m/e 210 (M+H), Calcd for C₁₁H₁₂ClNO, 209.

Step 3:

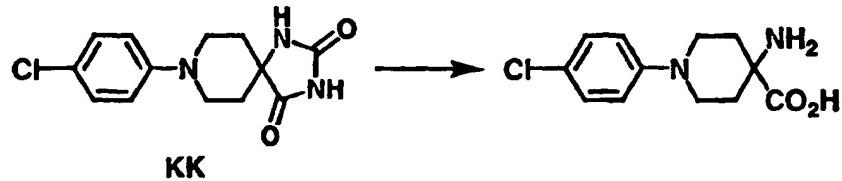
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To a solution of the ketone JJ (1.465 g, 6.986 mmole) in ethanol (75 mL) and water (25 mL) in a glass pressure bottle, were added ammonium carbonate (5.36 g, 55.88 mmole, 8 equiv.) and potassium cyanide (1.135 g, 17.46 mmol, 2.5 equiv.). The mixture was heated at 80-90 °C for 18 hrs. The cooled reaction mixture was concentrated in vacuo and the residue was treated 15 with water, extracted with EtOAc (4x). The combined organic extracts were washed with water, dried over anhydrous Na₂SO₄ and concentrated to give the spectroscopically pure hydantoin KK as a white solid (1.817 g, 93% yield). MS (electrospray) m/e 280 (M+H), Calcd for C₁₃H₁₄CIN₃O₂, 279.

Step 4:

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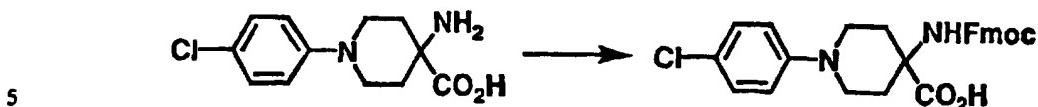


The hydantoin KK (1.768 g) was suspended in aqueous NaOH (6N, 50 mL) and heated at 130 °C for 4 days. Upon the completion (by HPLC) of the hydrolysis, the reaction mixture was neutralized with conc. HCl to slightly acidic (pH ~6). The resulting slurry was filtered, washed with water and dried to give 4-amino-1-(4-chlorophenyl)piperidine-4-carboxylic acid (4-25 CLAPPC) as a white solid (2.05 g, >100 % yield, wet and contaminated with inorganic salt),

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which showed a single peak on HPLC and used directly for the next step. MS (electrospray) *m/e* 253 (M-H), Calcd for C₁₂H₁₅ClN₂O₂, 254

Step 5:

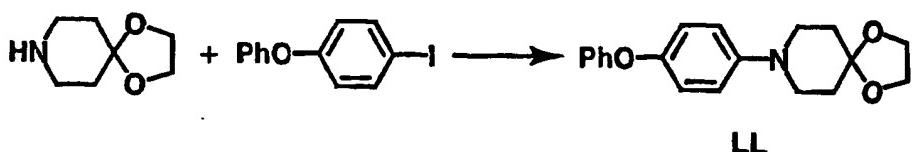


The crude 4-amino-1-(4-chlorophenyl)piperidine-4-carboxylic acid (4-ClAPPC) from the last step was suspended in dioxane (100 mL) and aqueous 10% Na₂CO₃ (50 ml), treated with Fmoc-Cl (2.0 g, 7.75 mmole, 1.2 equiv) and was stirred vigorously overnight. The reaction mixture was then concentrated to remove dioxane, neutralized with 6N HCl to slightly acidic (pH 6) and extracted with EtOAc. The combined organic extracts were washed with brine and dried over Na₂SO₄. Removal of the solvent gave the crude product which was purified on flash chromatography (hexane/EtOAc to CH₂Cl₂/MeOH) to give pure Fmoc-4-ClAPPC (1.18 g, 81% overall yield for two steps). ¹H NMR (DMSO-d₆): 7.87 (d, 2H), 7.71 (d, 2H), 7.39 (td, 2H), 7.30 (td, 2H), 7.20 (d, 2H), 6.92 (d, 2H), 3.44 (d, 2H), 2.93 (t, 2H); MS (electrospray) *m/e* 477 (M+H), Calcd for C₂₇H₂₅N₂O₄, 476.

EXAMPLE 20

Preparation of Fmoc-4-amino-1-(4-phenoxyphenyl)piperidine-4-carboxylic acid (Fmoc-4-PhOAppc-OH)

Step 1:

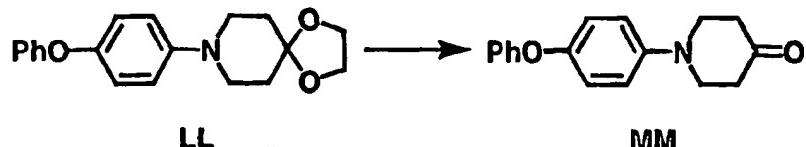


To a solution of 1-iodo-4-phenoxybenzene (3.15 g, 10.6 mmol), 1,4-dioxa-8-azaspiro [4.5] decane (3.3 mL, 3.66 g, 25.6 mmole, 2.4 equiv) and sodium tert-butoxide (2.85 g, 29.7 mmol, 2.8 equiv) in dry dioxane (40 mL) were added tris (dibenzylideneacetone) dipalladium (0) (48.5 mg, 0.053 mmol) and tri-*o*-tolyl- phosphine (64 mg, 0.4 mmol). The reaction was heated at 90 °C for 9 hrs. The resulting reaction mixture was concentrated to remove solvent.

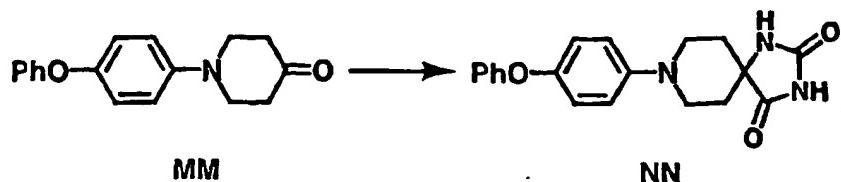
- 114 -

The residue was treated with water and extracted with EtOAc. The combined organic extracts were combined, washed with brine, dried over Na₂SO₄ and concentrated to give a brown oil. This crude product was purified on flash chromatography (hexane/EtOAc, 95/5 to 80/20) to provide the pure product LL as a slightly yellow solid (2.805, 85%). ¹H NMR (CDCl₃), 7.26-7.32 (m, 2H), 7.03 (t, 1H), 6.92-6.97 (m, 6H), 4.00 (s, 4H), 3.26 (t, 4H), 1.86 (t, 4H),

5

Step 2:

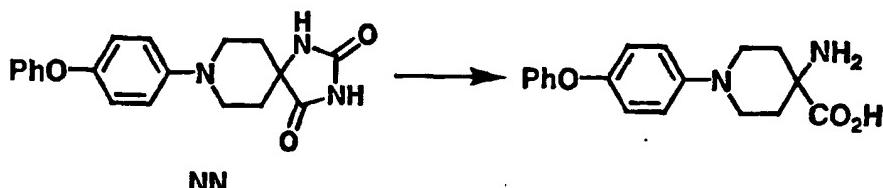
To a solution of the ketal LL (2.755 g, 8.86 mmol) in acetone (90 mL) was added 6N hydrochloric acid (45 mL) and the reaction was heated at reflux overnight. The resulting
10 reaction mixture was concentrated to remove solvent. The residue was diluted with EtOAc and neutralized with aqueous 6N NaOH. The layers were separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄ and concentrated to give the crude product which was purified on flash chromatography (hexane/EtOAc, 90/10 to 70/30) to give the product MM as a yellow oil (2.21 g, 93%). MS
15 (electrospray) *m/e* 268 (M+H), Calcd for C₁₇H₁₇ClNO₂, 267.

Step 3:

To a solution of the ketone MM (2.01 g, 7.52 mmol) in ethanol (80 mL) and water (25 mL) in a glass pressure bottle, were added ammonium carbonate (5.78 g, 60.0 mmol, 8 equiv.)
20 and potassium cyanide (1.22 g, 18.80 mmol, 2.5 equiv.). The mixture was heated at 80-90 °C for 18 hrs. The cooled reaction mixture was concentrated in vacuo and the residue was treated with water, extracted with EtOAc (4x). The combined organic extracts were washed with water, dried over anhydrous Na₂SO₄ and concentrated to give the spectroscopically pure hydantoin NN as a white solid (2.34 g, 95% yield). MS (electrospray) *m/e* 338 (M+H), Calcd for C₁₉H₁₉N₃O₃, 337.

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Step 4:



The hydantoin NN (2.28 g, 6.76 mmole) was suspended in aqueous NaOH (6N, 60 mL) and heated at 130 °C for 4 days. Upon completion (by HPLC) of the hydrolysis, the reaction
 5 mixture was neutralized with conc. HCl to slightly acidic (pH ~6). The resulting slurry was filtered, washed with water and dried to give 4-amino-1-(4-phenoxyphenyl)piperidine-4-carboxylic acid (4-PhOAPPC) as a white solid (2.53 g, >100 % yield, wet and contaminated with inorganic salt), which showed a single peak on HPLC and used directly for the next step. MS (electrospray) *m/e* 313 (M+H), Calcd for C₁₈H₂₀N₂O₃, 312.

10 Step 5:

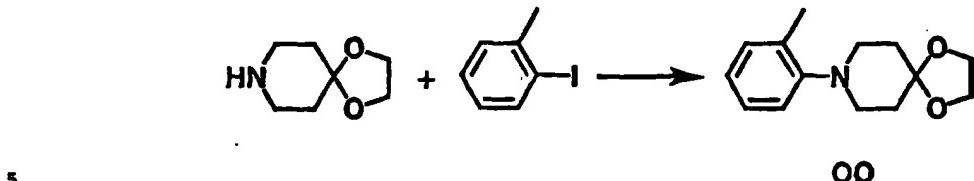


The crude 4-amino-1-(4-phenoxyphenyl)piperidine-4-carboxylic acid (4-PhOAPPC) from the last step was suspended in dioxane (50 L) and aqueous 10 % Na₂CO₃ (50 mL) and stirred vigorously overnight. The reaction mixture was concentrated to remove dioxane,
 15 neutralized with 6N HCl to slightly acidic (pH 6) and extracted with EtOAc. The combined organic extracts were washed with brine and dried over Na₂SO₄. Removal of the solvent gave the crude product which was purified on flash chromatography (hexane/EtOAc to CH₂Cl₂/MeOH) to give pure Fmoc-4-PhOAPPC (2.18 g, 60% overall yield for two steps). ¹H NMR (DMSO-d₆): 7.87 (d, 2H), 7.72 (d, 2H), 7.38 (t, 2H), 7.30 (td, 4H), 7.02 (dt, 1H), 6.86-6.96 (m, 6H), 3.35 (m, 2H), 2.94 (t, 2H); MS (electrospray) *m/e* 535 (M+H), Calcd for C₃₃H₃₀N₂O₅, 534.

EXAMPLE 21

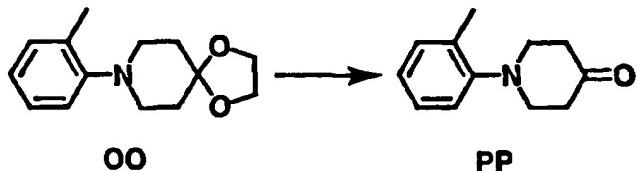
Preparation of Fmoc-4-amino-1-(2-methylphenyl)piperidine-4-carboxylic acid(Fmoc-2-MeAppc-OH)

Step 1:



- To a solution of 2-iodotoluene (4.36 g, 2.5 mL, 20.0 mmol), 1,4-dioxa-8-azaspiro[4.5]decane (6.88 g, 6.2 mL, 48.1 mmol, 2.4 equiv) and sodium tert-butoxide (5.3 g, 55.2 mmol, 2.8 equiv) in dry dioxane (80 mL) were added tris(dibenzylideneacetone)dipalladium(0) (91 mg, 0.1 mmol) and tri-*o*-tolylphosphine (122 mg, 0.4 mmol). The reaction was heated at 90
 10 °C for 26 hrs. The resulting reaction mixture was concentrated to remove solvent. The residue was treated with water and extracted with EtOAc. The combined organic extracts were combined, washed with brine, dried over Na₂SO₄ and concentrated to give brown oil. This crude product was purified on flash chromatography (hexane/EtOAc, 95/5 to 75/25) to provide the pure product OO as a slightly yellow solid (2.66 g, 57%). ¹H NMR (CDCl₃), 7.12-7.18 (m, 2H),
 15 6.94-7.06 (m, 2H), 4.01 (s, 4H), 2.98 (t, 4H) and 1.88 (t, 4H).

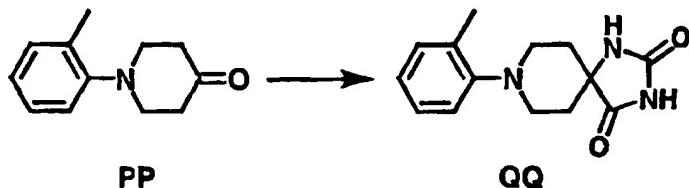
Step 2:



- To a solution of the ketal OO (2.66 g, 11.4 mmol) in acetone (70 mL) was added 6N hydrochloric acid (35 mL) and the reaction was heated at 85 °C overnight. The resulting
 20 reaction was concentrated to remove solvent. The residue was diluted with EtOAc and neutralized with aqueous NaOH (6N). The layers were separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄ and concentrated. The crude product was purified on flash chromatography (hexane/EtOAc, 90/10 to 70/30) to give the product PP as a yellow oil (2.04 g, 95%). MS
 25 (electrospray) *m/e* 190 (M+H), Calcd for C₁₂H₁₅NO, 189

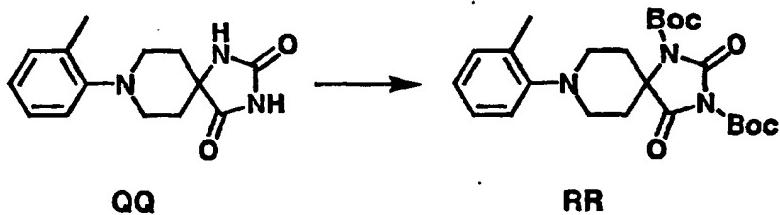
- 117 -

Step 3:



To a solution of the ketone PP (1.54 g, 8.15 mmol) in ethanol (60 mL) and water (20 mL) in a glass pressure bottle, were added ammonium carbonate (4.69 g, 48.9 mmol, 6 equiv.) and 5 potassium cyanide (800 g, 12.2 mmol, 1.5 equiv.). The mixture was heated at 80-90 °C for 18 hrs. The cooled reaction mixture was added to icy water (300 ml) and stirred vigorously for 30 min. The resulting precipitate was suction filtered, washed thoroughly with water and dried to yield the hydantoin QQ as a white solid (2.01 g, 95% yield). MS (electrospray) *m/e* 260 (M+H), Calcd for C₁₄H₁₇N₃O₂, 259

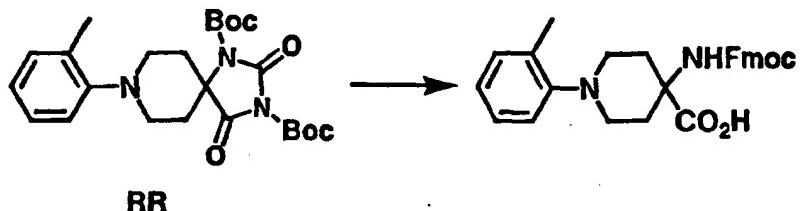
10 Step 4:



To a suspension of the hydantoin QQ (1.07 g, 4.13 mmol) in dry THF (25 mL) were added di-*tert*-butyl dicarbonate (2.25 g, 10.32 mmol, 2.5 equiv), triethylamine (0.63 mL, 460 mg, 4.54 mmol, 1.1 equiv) and DMAP (36 mg, 0.29 mmol) in succession. About 15 minutes after the 15 addition, the reaction turned into a clear yellow solution and was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to yield a solid that was then taken up in EtOAc (300 mL), washed with 1N HCl (3x30 mL), saturated aqueous Na₂CO₃ (2x30 mL) and brine (2x30 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude light yellow product was purified through flash chromatography 20 (hexane/EtOAc, 90/10→80/20) to give the pure bis-Boc hydantoin RR as a white solid (1.71 g, 90%). MS (electrospray) *m/e* 460 (M+H), Calcd for C₂₄H₃₃N₃O₆, 459

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Step 5:

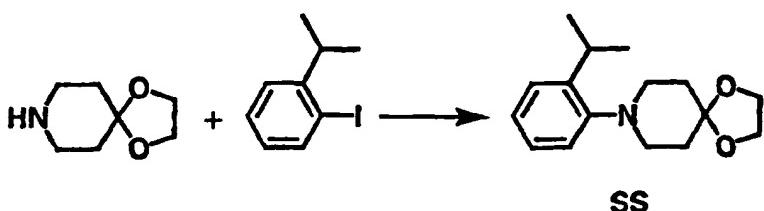


The bis-Boc hydantoin RR (1.71g, 3.72 mmol) was dissolved in DME (23 mL) to give a clear solution. To this solution was added 1N NaOH (33 mL, 33 mmol) and the reaction was stirred overnight at room temperature, giving a fairly clear mixture. HPLC showed completion of the reaction. The reaction mixture was concentrated under reduced pressure to remove DME and extracted with Et₂O. Without purification, the resulting aqueous layer containing 4-amino-1-(2-methylphenyl)piperidine-4-carboxylic acid (2-MeAPPC) was treated with 6N HCl to adjust the pH to 11-12. This solution (30 mL) was then diluted with 1,4-dioxane (30 mL) and treated with 10 Fmoc-Cl (1.28 g, 4.96 mmol, 1.3 equiv) and stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to remove dioxane, neutralized with 1N HCl and extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified through flash chromatography (hexane/EtOAc → CH₂Cl₂/MeOH) to give the pure product Fmoc-2-MeAPPC 15 as a white solid (1.09 g, 64 % yield from the bis-Boc hydantoin RR). ¹H NMR (DMSO-d₆): 7.87 (d, 2H), 7.74 (d, 2H), 7.40 (td, 2H), 7.31 (td, 2H), 7.12 (m, 2H), 6.97 (d, 1H), 6.92 (td, 1H), 2.72-2.88 (m, 4H) and 2.22 (s, 3H); MS (electrospray) *m/e* 457 (M+H), Calcd for C₂₈H₂₈N₂O₄, 456.

20 EXAMPLE 22

Preparation of Fmoc-4-amino-1-(2-isopropylphenyl)piperidine-4-carboxylic acid (Fmoc-2-iPrAppc-OH)

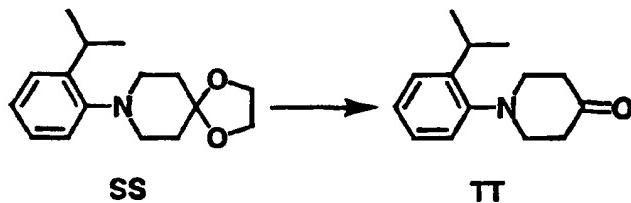
Step 1:



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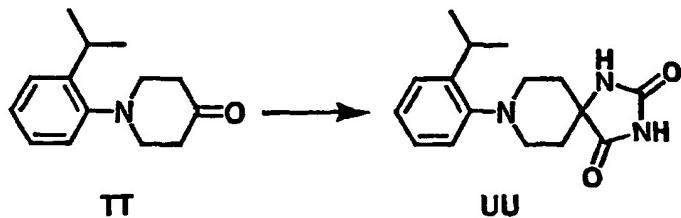
To a solution of 1-iodo-2-*iso*-propylbenzene (10.0 g, 40.7 mmol), 1,4-dioxa-8-azaspiro[4.5]decane (12.0 mL, 13.3 g, 93.0 mmol, 2.3 equiv) and sodium tert-butoxide (10.0 g, 104.2 mmol, 2.6 equiv) in dry dioxane (160 mL) were added tris(dibenzylideneacetone)dipalladium(0) (180 mg, 0.197 mmol) and tri-*o*-tolyl-phosphine (244 mg, 0.80 mmol) and the reaction was heated at 90 °C for 26 hrs. The resulting reaction mixture was concentrated to remove solvent, treated with water and extracted with EtOAc. The combined organic extracts were combined, washed with brine, dried over Na₂SO₄ and concentrated to give a brown oil. This crude product was purified on flash chromatography (hexane/EtOAc, 95/5 → 75/25) to provide the pure product SS as a slightly yellow solid (3.61 g, 10 35% yield). MS m/z 262 (M+H), Calcd for C₁₆H₂₃NO₂, 261.

Step 2:



To a solution of the ketal SS (3.24 g, 12.4 mmol) in acetone (90 mL) was added 6N hydrochloric acid (45 mL) and the reaction was heated at reflux overnight. The resulting 15 reaction mixture was concentrated to remove solvent and the residue was diluted with EtOAc, neutralized with aqueous NaOH (6N). The layers were separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄ and concentrated. The crude product was purified on flash chromatography (hexane/EtOAc, 90/10 → 70/30) to give the product TT as a yellow oil (2.42 g, 89%). ¹H NMR (CDCl₃): 7.27 (m, 1H), 7.04-7.19 (m, 3H), 3.58 (m, 1H), 3.20 (t, 4H), 2.60 (t, 4H) and 1.25 (d, 6H); MS m/z 218 (M+H), Calcd for C₁₄H₁₉NO, 217

Step 3:

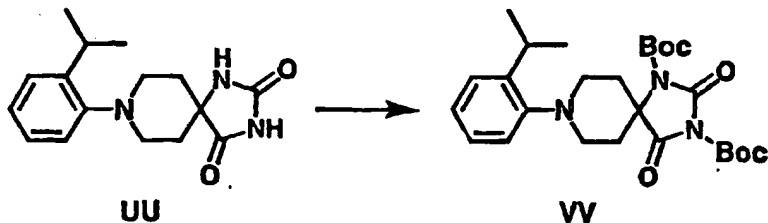


To a solution of the ketone TT (2.30 g, 10.6 mmol) in ethanol (90 mL) and water (20 mL) 25 in a glass pressure bottle, were added ammonium carbonate (8.1 g, 84.3 mmol, 8 equiv) and

- 120 -

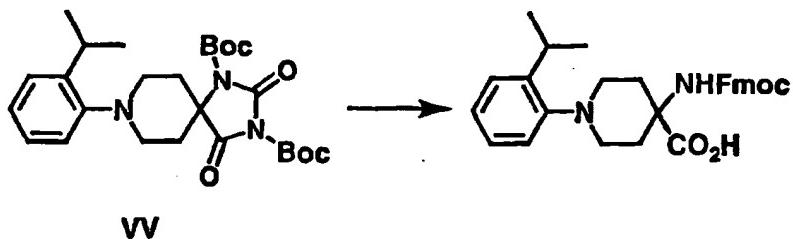
potassium cyanide (1.72 g, 26.5 mmol, 2.5 equiv). The mixture was heated at 80-90°C for 18 hrs. The cooled reaction mixture was added to icy water (400 ml) and stirred vigorously for 30 min. The resulting precipitate was suction filtered, washed thoroughly with water and dried to yield the hydantoin UU as a white solid (2.78 g, 91% yield). MS m/z 288 (M+H), Calcd for 5 $C_{16}H_{21}N_3O_2$, 287.

Step 4:



- To a suspension of the hydantoin UU (2.74 g, 9.54 mmol) in dry THF (100 mL) were added di-*tert*-butyl dicarbonate (5.2 g, 24.24 mmol, 2.5 equiv), triethylamine (1.5 mL, 1.07 g, 10.5 mmol, 1.1 equiv) and DMAP (46 mg, 0.29 mmol) in succession. About 15 minutes after the addition, the reaction turned into a clear yellow solution and was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to yield a solid that was then taken up in EtOAc (300 mL), washed with brine (3x30 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude light yellow product was purified 15 through flash chromatography (hexane/EtOAc, 90/10 → 80/20) to give the pure bis-Boc hydantoin VV as a white solid (4.39 g, 94% yield). MS m/z 488 (M+H), Calcd for C₂₆H₃₇N₃O₆, 487.

Step 5:



- 20 The bis-Boc hydantoin VV (2.34g, 4.8 mmol) was dissolved in DME (30 mL) to give a clear solution. To this solution was added 1N NaOH (45 mL, 45 mmol) and the reaction was stirred overnight at room temperature, giving a fairly clear mixture. HPLC showed completion of the reaction. The reaction mixture was concentrated under reduced pressure to remove DME and extracted with Et₂O. Without purification, the resulting aqueous layer containing 4-amino-1-(2-

- 121 -

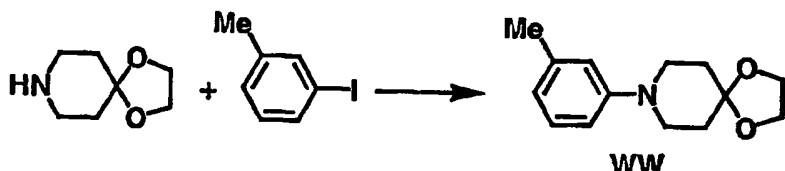
isopropylphenyl)piperidine-4-carboxylic acid (2-iPrAPPC) was treated with 6N HCl to adjust the pH to 11-12. This solution (~45 mL) was then diluted with 1,4-dioxane (45 mL) and treated with Fmoc-Cl (1.78 g, 6.89 mmol, 1.5 equiv) and stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to remove dioxane, neutralized with 5 1N HCl and extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified through flash chromatography (hexane/EtOAc → CH₂Cl₂/MeOH) to give the pure product Fmoc-2-iPrAPPC as a white solid (1.46 g, 63 % yield from the bis-Boc hydantoin). HRMS m/z 507.2263, Calcd for C₃₀H₃₂N₂O₄Na, 507.2260.

10

EXAMPLE 23

Preparation of Fmoc-4-amino-1-(3-methylphenyl)piperidine-4-carboxylic acid (Fmoc-3-MeAppc-OH)

15 Step 1:

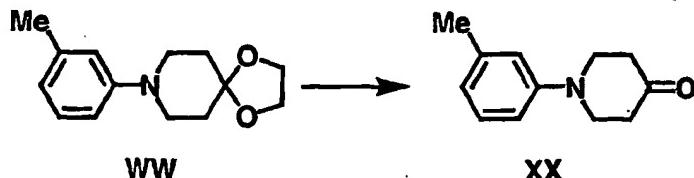


To a solution of 3-iodotoluene (4.36 g, 2.6 mL, 20.0 mmol), 1,4-dioxa-8-azaspiro [4.5] decane (6.88 g, 6.2 mL, 48.1 mmol, 2.4 equiv) and sodium tert-butoxide (5.3 g, 55.2 mmol, 2.8 equiv) in dry dioxane (80 mL) were added tris (dibenzylideneacetone) dipalladium (0) (91 mg, 0.1 mmol) and tri-*o*-tolylphosphine (122 mg, 0.4 mmol). The reaction was heated at 90 °C for 26 hrs. The resulting reaction mixture was concentrated to remove solvent. The residue was treated with water and extracted with EtOAc. The combined organic extracts were combined, washed with brine, dried over Na₂SO₄ and concentrated to give a brown oil. This crude product was purified on flash chromatography (hexane/EtOAc, 95/5 to 75/25) to provide the pure product 20 WW as a slightly yellow solid (3.21 g, 69%).

25 WW as a slightly yellow solid (3.21 g, 69%).

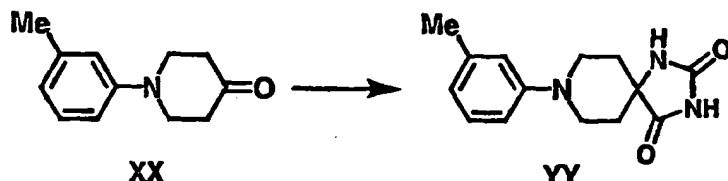
- 122 -

Step 2:



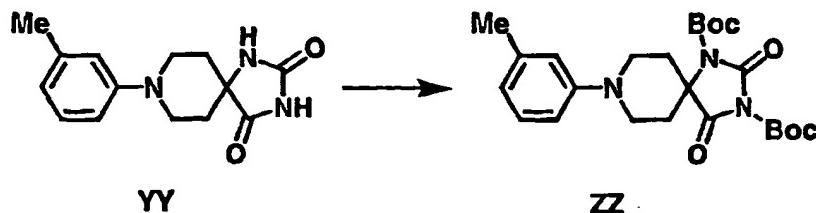
To a solution of the ketal WW (1.25 g, 5.36 mmol) in acetone (20 mL) was added 6N hydrochloric acid (10 mL) and the reaction was heated at reflux overnight. The resulting 5 reaction was concentrated to remove solvent. The residue was diluted with EtOAc and neutralized with aqueous NaOH (6N). The layers were separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄ and concentrated. The crude product was purified on flash chromatography (hexane/EtOAc, 90/10 to 70/30) to give the product XX as a yellow oil (843 mg, 83% yield). MS 10 m/z 190 (M+H), Calcd for C₁₂H₁₅NO, 189.

Step 3:



To a solution of the ketone XX (763 g, 4.03 mmol) in ethanol (45 mL) and water (15 mL) in a glass pressure bottle, were added ammonium carbonate (3.09 g, 32.21 mmol, 8 equiv) and 15 potassium cyanide (675 mg, 10.38 mmol, 2.5 equiv). The mixture was heated at 80-90 °C for 18 hrs. The cooled reaction mixture was added to icy water (200 ml) and stirred vigorously for 30 min. The resulting precipitate was suction filtered, washed thoroughly with water and dried to yield the hydantoin YY as a white solid (930 mg, 89% yield). MS m/z 260 (M+H), Calcd for C₁₄H₁₇N₃O₂, 259.

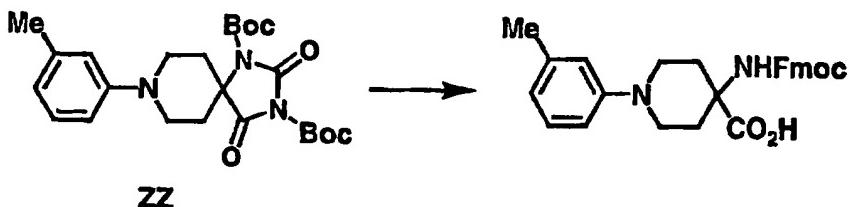
20 Step 4:



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To a suspension of the hydantoin YY (780 mg, 3.012 mmol) in dry THF (22 mL) were added di-*tert*-butyl dicarbonate (1.64 g, 7.52 mmol, 2.5 equiv), triethylamine (0.42 mL, 305 mg, 3.01 mmol, 1.0 equiv) and DMAP (20 mg, 0.164 mmol) in succession. About 5 minutes after the addition, the reaction turned into a clear yellow solution and was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to yield a solid that was then taken up in EtOAc (300 mL), washed with brine (3x30 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude light yellow product was purified through flash chromatography (hexane/EtOAc, 90/10 → 80/20) to give the pure bis-Boc hydantoin ZZ as a white solid (1.37 g, quantitative). HRMS m/z 482.2261 (M+Na), Calcd. for C₂₄H₃₃N₃O₆Na, 482.2267.

Step 5:



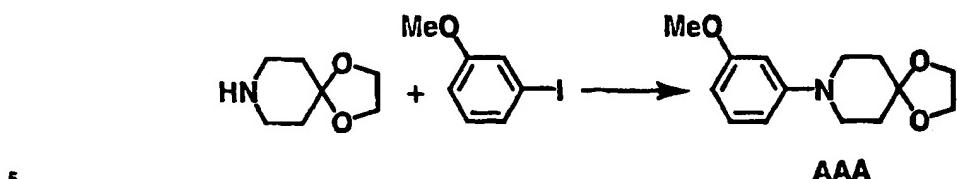
The bis-Boc hydantoin ZZ (1.29 g, 2.818 mmol) was dissolved in DME (20 mL) to give a clear solution. To this solution was added 1N NaOH (25 mL, 25 mmol) and the reaction was stirred overnight at room temperature, giving a fairly clear mixture. HPLC showed completion of the reaction. The reaction mixture was concentrated under reduced pressure to remove DME and extracted with Et₂O. Without purification, the resulting aqueous layer containing 4-amino-1-(3-methylphenyl)piperidine-4-carboxylic acid (3-MeAPPC) was treated with 6N HCl to adjust the pH to 11-12. This solution (30 mL) was then diluted with 1,4-dioxane (30 mL) and treated with Fmoc-Cl (1.46 mg, 5.65 mmol, 2.0 equiv) and stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to remove dioxane, neutralized with 1N HCl and extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified through flash chromatography (hexane/EtOAc → CH₂Cl₂/MeOH) to give the pure product Fmoc-3-MeAPPC as a white solid (1.002 g, 78 % yield from the bis-Boc hydantoin). HRMS m/z 479.1940 (M+Na), Calcd. for C₂₈H₂₈N₂O₄Na, 479.1947.

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EXAMPLE 24

Preparation of Fmoc-4-amino-1-(3-methoxyphenyl)piperidine-4-carboxylic acid (Fmoc-3-MeOAppc-OH)

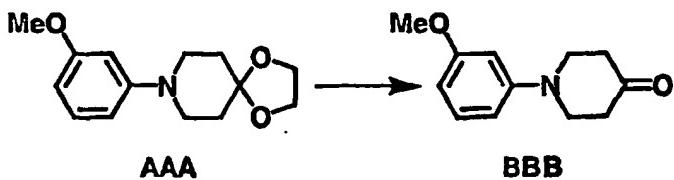
Step 1:



5

To a solution of 3-iodoanisole (4.68 g, 2.4 mL, 20.0 mmol), 1,4-dioxa-8-azaspiro [4.5] decane (6.2 mL, 6.88 g, 48.1 mmol, 2.4 equiv) and sodium tert-butoxide (5.3 g, 55.2 mmol, 2.8 equiv) in dry dioxane (80 mL) were added tris(dibenzylideneacetone)dipalladium(0) (91 mg, 0.1 mmol) and tri-*o*-tolylphosphine (122 mg, 0.4 mmol) and the reaction was heated at 90 °C for 26 hrs. The resulting reaction mixture was concentrated to remove solvent and the residue was treated with water and extracted with EtOAc. The combined organic extracts were combined, washed with brine, dried over Na₂SO₄ and concentrated to give brown oil. This crude product was purified on flash chromatography (hexane/EtOAc, 95/5 to 75/25) to provide the pure product AAA as a slightly yellow solid (3.10 g, 62% yield). MS *m/z* (M+H), 250 (M+H), Calcd for C₁₄H₁₉NO₃, 249.

Step 2:

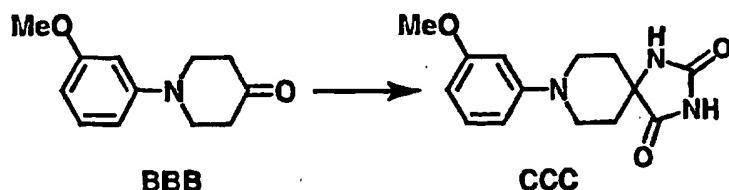


To a solution of the ketal AAA (3.10 g, 12.45 mmol) in acetone (90 mL) was added 6N hydrochloric acid (45 mL) and the reaction was heated at reflux overnight. The resulting reaction was concentrated to remove solvent. The residue was diluted with EtOAc and neutralized with aqueous NaOH (6N). The layers were separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine, dried over Na₂SO₄ and concentrated. The crude product was purified on flash chromatography (hexane/EtOAc, 90/10 to 70/30) to give the product BBB as a yellow oil (2.53 g, 99% yield). ¹H

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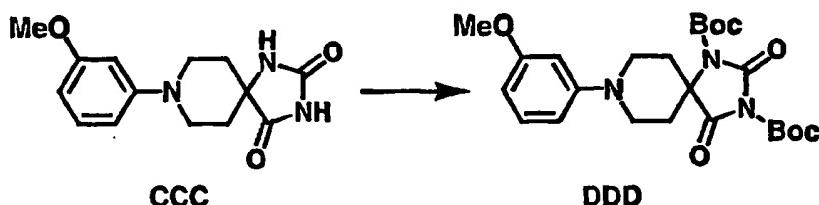
NMR (CDCl_3): 7.20 (m, 1H), 6.58 (d, 1H), 6.39-6.56 (m, 2H), 3.80 (s, 3H), 3.59 (m, 4H) and 2.58 (m, 4H).

Step 3:



5 To a solution of the ketone BBB (1.81 g, 8.82 mmol) in ethanol (60 mL) and water (20 mL) in a glass pressure bottle, were added ammonium carbonate (6.77 g, 70.52 mmol, 8 equiv) and potassium cyanide (1.14g, 17.6 mmol, 2.0 equiv). The mixture was heated at 80-90 °C for 18 hrs. The cooled reaction mixture was added to icy water (200 ml) and stirred vigorously for 30 min. The resulting precipitate was suction filtered, washed thoroughly with water and dried to 10 yield the hydantoin CCC as a white solid (2.23 g, 92% yield). MS m/z 276 (M+H), Calcd for $\text{C}_{14}\text{H}_{17}\text{N}_3\text{O}_3$, 275.

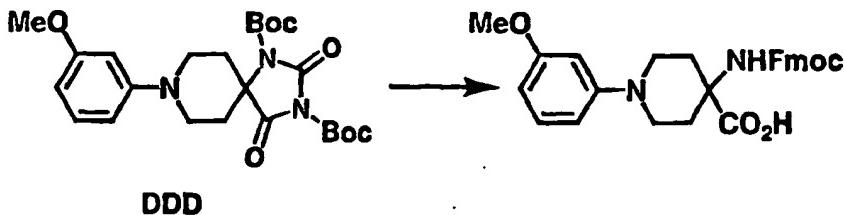
Step 4:



To a suspension of the hydantoin CCC (1.10 g, 4.00 mmol) in dry THF (50 mL) were 15 added di-*tert*-butyl dicarbonate (2.18 g, 10.0 mmol, 2.5 equiv), triethylamine (0.62 mL, 445 mg, 4.4 mmol, 1.1 equiv) and DMAP (20 mg, 0.164 mmol) in succession. About 15 minutes after the addition, the reaction turned into a clear yellow solution and was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to yield a solid that was then taken up in EtOAc (300 mL), washed with brine (3x30 mL), dried over anhydrous 20 Na_2SO_4 and concentrated under reduced pressure. The crude light yellow product was purified through flash chromatography (hexane/EtOAc, 90/10 → 80/20) to give the pure bis-Boc hydantoin DDD as a white solid (1.90 g, quantitative), ^1H NMR (CDCl_3): 7.16 (t, 1H), 6.57 (d, 1H), 6.24 (s, 1H), 6.19 (d, 1H), 3.77 (s, 3H), 1.58 (s, 9H), 1.42 (s, 9H); MS m/z 476 (M+H), Calcd for $\text{C}_{24}\text{H}_{33}\text{N}_3\text{O}_7$, 475.

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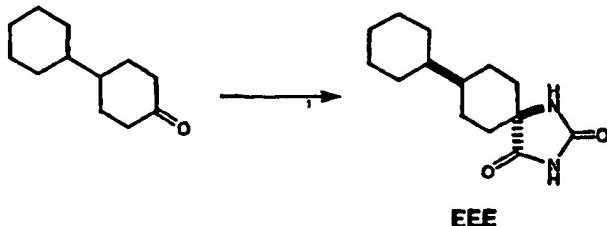
Step 5:



The bis-Boc hydantoin DDD (1.06 g, 2.23 mmol) was dissolved in DME (20 mL) to give a clear solution. To this solution was added 1N NaOH (20 mL, 20 mmol) and the reaction was stirred overnight at room temperature, giving a fairly clear mixture. HPLC showed completion of the reaction. The reaction mixture was concentrated under reduced pressure to remove DME and extracted with Et₂O. Without purification, the resulting aqueous layer containing 4-amino-1-(3-methoxyphenyl)piperidine-4-carboxylic acid (3-MeOAPPC) was treated with 6N HCl to adjust the pH to 11-12. This solution (35 mL) was then diluted with 1,4-dioxane (35 mL) and treated with Fmoc-Cl (755 mg, 2.93 mmol, 1.3 equiv) and stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure to remove dioxane, neutralized with 1N HCl and extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified through flash chromatography (hexane/EtOAc → CH₂Cl₂/MeOH) to give the pure product Fmoc-3-MeOAPPC as a white solid (668 mg, 63 % yield from the bis-Boc hydantoin DDD). ¹H NMR (CDCl₃): 7.83 (d, 2H), 7.72 (d, 2H), 7.41 (td, 2H), 7.34 (dt, 2H), 7.16 (t, 1H), 6.52 (d, 1H), 6.42 (s, 1H), 6.36 (d, 1H), 4.25 (m, 3H), 3.68 (s, 3H), 3.23-3.40 (m, 2H), 2.96 (t, 2H) and 1.86-2.18 (m, 4H). HRMS m/z 495.1901 (M+Na), Calcd. for C₂₈H₂₈N₂O₅Na, 495.1896.

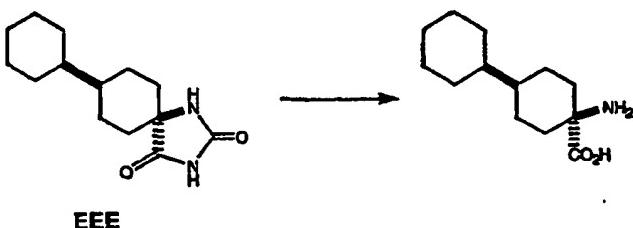
20 EXAMPLE 25**Preparation of Fmoc-1-amino-4-cyclohexylcyclohexane-1-carboxylic acid (Fmoc-Achc-OH)**

Step 1:

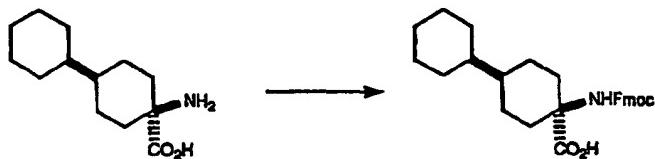


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A mixture of 4-cyclohexylcyclohexanone (3.00 g, 16.6 mmole), potassium cyanide (1.63 g, 25.0 mmole), ammonium carbonate (9.59 g, 99.8 mmole), ethanol (75 ml) and water (15 ml) in a sealed, thick walled pressure flask was heated in a 80°C oil bath for 15 hours. After cooling to room temperature, the white slurry was poured into ice-water and stirred at room temperature 5 for a couple of hours. Filtration and air-drying gave hydantoin EEE (6.10 g, still wet, >100% yield) as a white solid. ^1H NMR (DMSO-d₆) δ 10.52 (1H, broad, NH), 8.43 (1H, broad s, NH), 0.80-1.80 (20H, m). LRMS (APCI): C₁₄H₂₂N₂O₂, calc. 250; observed: 249 (M-H), 251 (M+H).

Step 2:

10 A mixture of hydantoin EEE (1.39 g, 5.55 mmole) and 6N sodium hydroxide solution (50 ml) in a sealed, thick walled pressure flask was heated in a 130°C oil bath for 2 days. The reaction mixture was cooled in an ice bath, neutralized to ~ pH 7 using concentrated hydrochloric acid. The white slurry was filtered and the precipitates rinsed with water to give crude 1-amino-4-cyclohexylcyclohexane-1-carboxylic acid (48.3 g, wet and containing 15 inorganic salts, >100% yield). LRMS (Electrospray): C₁₃H₂₃NO₂, calc. 225; observed: 226 (M+H).

Step 3:

20 A mixture of crude 1-amino-4-cyclohexylcyclohexane-1-carboxylic acid (48.3 g, 5.55 mmole theoretical), triethylamine (1.0 ml, 7.17 mmole), 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu, 2.43 g, 7.20 mmole) in acetonitrile (75 ml) and water (75 ml) was stirred at room temperature for 24 hours. The reaction mixture was concentrated in vacuo to remove most of the acetonitrile, acidified to pH ~3 with 10% aqueous citric acid solution, and the white 25 emulsion extracted three times with methylene chloride. The combined organic layers were washed with water, brine, dried over magnesium sulfate. Filtration and concentration gave a crude oil which was purified by column chromatography (eluted with 1 → 5 → 8%

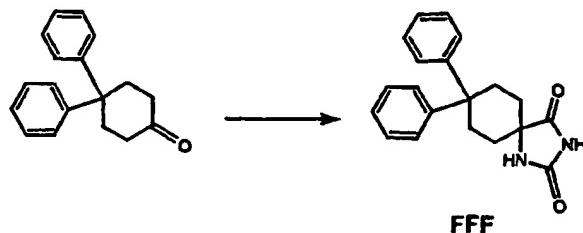
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methanol/methylene chloride) to give Fmoc-1-amino-4-*trans*-cyclohexylcyclohexane-1-carboxylic acid (250 mg, 10% yield for two steps). HRMS (FAB): C₂₈H₃₄NO₄ (M+H) calc. 448.2488; observed: 448.2497.

5 EXAMPLE 26

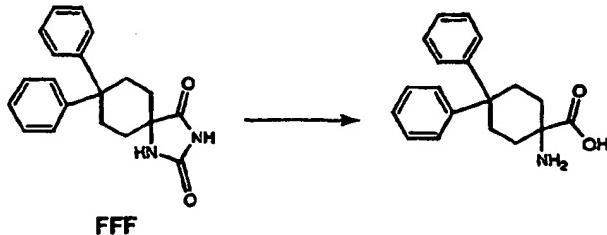
Preparation of Fmoc-1-amino-4,4-diphenylcyclohexane-1-carboxylic acid (Fmoc-Adpc-OH)

Step 1:



- A mixture of 4,4-diphenylcyclohexanone (prepared by hydrogenation of 4,4-diphenylcyclohexenone according to the procedures of Freeman, P.K. et.al. *J. Org. Chem.* 1989, 54, 782-789) (1.55 g, 6.19 mmole), potassium cyanide (0.65 g, 9.97 mmole), ammonium carbonate (3.60 g, 37.5 mmole), ethanol (48 ml) and water (12 ml) in a sealed, thick walled pressure flask was heated in a 80°C oil bath for 24 hours. After cooling to room temperature, the white slurry was poured into ice-water and stirred at room temperature for a couple of hours.
- Filtration and air-drying gave hydantoin FFF (1.89 g, 95% yield) as a white solid. ¹H NMR (DMSO-d₆) δ 10.57 (1H, broad, NH), 8.59 (1H, broad s, NH), 7.00-7.50 (10H, m, phenyl). LRMS (Electrospray): C₂₀H₂₀N₂O₂, calc. 320; observed: 319 (M-H).

Step 2:



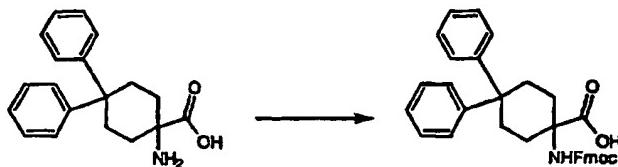
- A mixture of hydantoin FFF (1.88 g, 5.87 mmole), barium hydroxide monohydrate (5.60 g, 29.6 mmole) and water (100 ml, too dilute!) in a sealed, thick walled pressure flask was heated in a 105°C oil bath for 2 days. More barium hydroxide monohydrate (5.60 g, 29.6 mmole) was

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- added and the mixture was heated in a 105°C oil bath for another 24 hours. The reaction mixture was cooled to room temperature, acidified to ~ pH 3 using 4N sulfuric acid while being stirred vigorously. The suspension was stirred in a boiling water bath for two hours and cooled to room temperature. The white suspension was filtered and the precipitates rinsed with water.
- 5 The combined filtrate and washings were concentrated in vacuo to ~ 30 ml. Neutralization with concentrated ammonium hydroxide solution gave white precipitates which were filtered, washed with water and dried in vacuo overnight to give crude 1-amino-4,4-diphenylcyclohexane-1-carboxylic acid (0.52 g, 30% yield) as a white solid. LRMS (Electrospray): C₁₉H₂₁NO₂, calc. 295; observed: 294 (M-H), 296 (M+H).

10

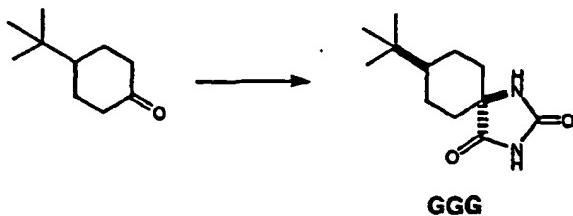
Step 3:



- 15 A mixture of crude 1-amino-4,4-diphenylcyclohexane-1-carboxylic acid (510 mg, 1.73 mmole), triethylamine (0.37 ml, 2.65 mmole), 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu, 880 mg, 2.61 mmole) in acetonitrile (25 ml) and water (25 ml) was stirred at room temperature overnight. TLC analysis of the reaction indicated the presence of starting material amino acid. 9-fluorenylmethyl succinimidyl carbonate (200 mg) and acetonitrile (5 ml) were
- 20 added and the mixture was stirred at room temperature for another 24 hours. The reaction mixture was concentrated in vacuo to remove most of the acetonitrile, acidified to pH ~3 with 10% aqueous citric acid solution, and the white emulsion extracted three times with ethyl acetate. The combined organic layers were washed with water, brine, dried over sodium sulfate. Filtration and concentration gave a crude oil which was purified by column chromatography
- 25 (eluted with 1 → 4 → 8% methanol/methylene chloride) to give Fmoc-1-amino-4,4-diphenylcyclohexane-1-carboxylic acid (350 mg, 39% yield) as a white solid. HRMS (FAB): C₃₄H₃₂NO₄ (M+H) calc. 518.2331; observed: 518.231

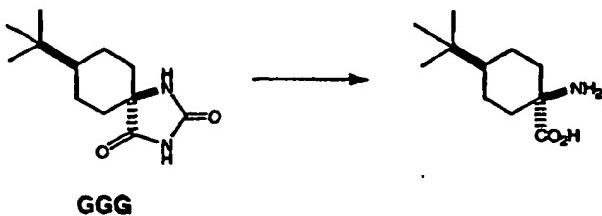
EXAMPLE 27Preparation of Fmoc-1-amino-4-*trans*-*t*-butylcyclohexane-1-carboxylic acid (Fmoc-Abc-OH)

Step 1:



- 5 A mixture of 4-*t*-butylcyclohexanone (2.00 g, 13.0 mmole), potassium cyanide (1.27 g, 19.5 mmole), ammonium carbonate (7.48 g, 77.8 mmole), ethanol (60 ml) and water (12 ml) in a sealed, thick walled pressure flask was heated in a 80°C oil bath for 15 hours. After cooling to room temperature, the white slurry was poured into ice-water and stirred at room temperature for a couple of hours. Filtration gave hydantoin GGG (2.78 g, 96% yield) as a white solid which
10 was used in the next step as a crude. ^1H NMR (DMSO-d₆) δ 10.52 (1H, broad, NH), 8.50 (1H, broad s, NH), 0.81 (9H, s, *t*-Bu).

Step 2:



- A mixture of hydantoin GGG (2.78 g, 12.4 mmole), barium hydroxide monohydrate
15 (11.74 g, 62.0 mmole) and water (50 ml) in a sealed, thick walled pressure flask was heated in a 120°C oil bath for 2 days. The reaction mixture was cooled to room temperature, acidified to ~ pH 3 using 4N sulfuric acid while being stirred vigorously. The suspension was stirred in a boiling water bath for one hour and cooled to room temperature. The white suspension was filtered and the precipitates rinsed with water. The combined filtrate and washings were
20 concentrated in vacuo to ~ 30 ml. Neutralization with concentrated ammonium hydroxide solution gave white precipitates which were filtered, washed with water and dried in vacuo overnight to give 1-amino-4-*trans*-*t*-butylcyclohexane-1-carboxylic acid (2.10 g, 85% yield) as a white solid.

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Step 3:

5 A mixture of crude 1-amino-4-*trans*-*t*-butylcyclohexyl-1-carboxylic acid (2.10 g, 10.54 mmole), 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu, 6.33 g, 7.20 mmole) in dioxane (150 ml) and 10% sodium carbonate solution (120 ml) was stirred at room temperature for 24 hours. The reaction mixture was concentrated in vacuo to remove most of the dioxane, acidified to pH ~3 with 3N HCl, and the white emulsion extracted twice with methylene chloride. The 10 combined organic layers were washed with water, brine, dried over magnesium sulfate. Filtration and concentration gave a crude which was purified by column chromatography (eluted with 1 → 4 → 5% methanol/methylene chloride) to give Fmoc-1-amino-4-*trans*-*t*-butylcyclohexane-1-carboxylic acid (1.42 g, 32% yield). HRMS (FAB): C₂₆H₃₂NO₄ (M+H) calc. 422.2331; observed: 422.23

15

EXAMPLE 28

Preparation of 3*S*, 2*S*-Fmoc-(L)-beta methyl (Nin-Mes) tryptophan, Fmoc-(L)-β-Me(Nin-Mes)Trp-OH)

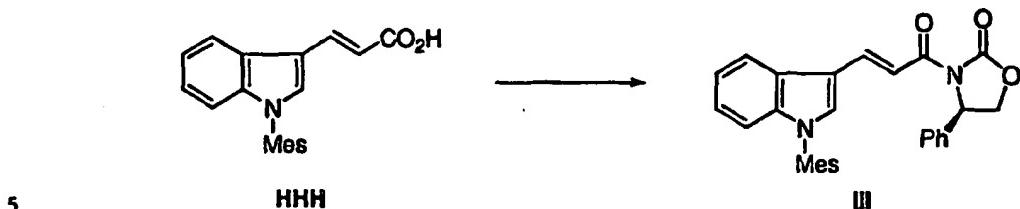
20 **Step 1:**

To a solution of trans-3-indolecarboxylic acid (15.0 g, 0.08 mole) in 350 mL of dry THF at -78 °C was slowly added 125 mL of 1.6 M n-BuLi in hexane. The resulting suspension was stirred at -78 °C for 1 hr. Then, a solution of 2-mesitylenesulfonyl chloride (21.9 g, 0.1 mole) in 50 mL of dry THF was slowly added. The mixture was warmed to rt, and stirred overnight. The mixture was poured to a saturated aqueous NH₄Cl solution. The layers were separated and the aqueous layer was extracted with EtOAc. The combined organic layer was dried over sodium sulfate. Removal of solvents gave 14.1 g of crude product HHH which was used for the next step without

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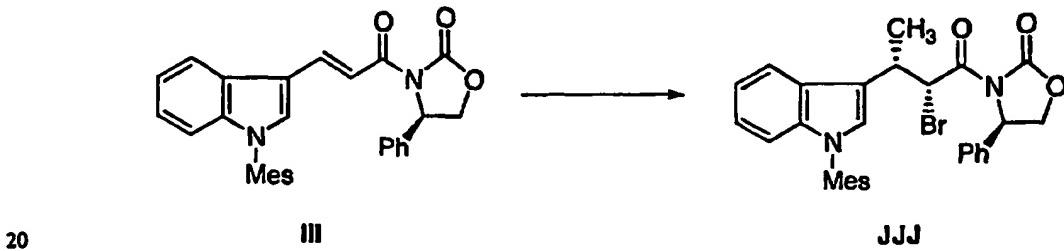
further purification. ^1H NMR analysis indicates that it contains 2.8 g of 2-mesitylenesulfonic acid. ^1H NMR (CD_3OD) δ 7.57 (s, 1H), 7.42 (d, 1H), 7.15-7.30 (m, 3H), 7.02 (s, 2H), 6.54 (d, 1H), 6.36 (d, 1H), 2.52 (s, 9H), 2.30 (s, 3H).

Step 2:



To a solution of N-2-mesitylenesulfonyl-trans-3-indolearylic acid (3.26 g, 8.8 mmole) in 140 mL of dry THF at -78 °C were added 3.7 mL (3 equiv.) of triethylamine and 2.17 mL (2 equiv.) of Me₃CCOCl. The resulting mixture was stirred at -78 °C for 15 min, and at 0 °C for 1.5 hr. The mixture was cooled down to -78 °C, to which was added 5.5 mL of 1.6M n-BuLi in hexane, then added a mixture of (R)-4-phenyl-2-oxazolidinone and n-BuLi in THF (prepared by adding 11 mL of 1.6M n-BuLi in hexane to a solution of (R)-4-phenyl-2-oxazolidinone (2.87 g, 17.6 mmole) in 70 mL of dry THF at -78 °C) through a cannula. The resulting mixture was stirred at -78 °C for 2 hr, and at rt overnight. The reaction was quenched by NH₄Cl aqueous solution (100 mL). After removal of the organic solvents in vacuo, the aqueous residue was extracted with EtOAc. The combined organic layer was dried over sodium sulfate. Filtration and concentration gave a crude product which was purified by a flash chromatography (EtOAc/hexane, 1:4) to give the product III as light brown gum in 63% yield (2.86 g). LR-Electrospray: C₂₉H₂₆N₂O₅S, cal: 514 observed: m/z 515 (M+H).

Step 3:

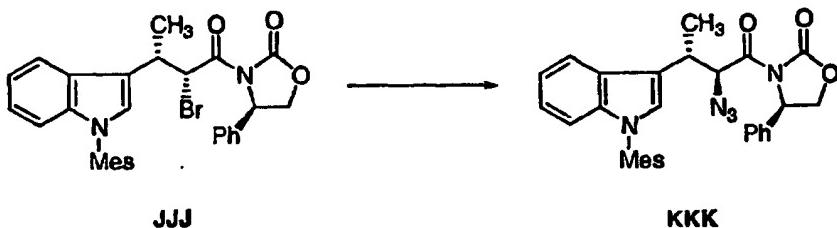


To a mixture of $\text{CuBr} \cdot \text{Me}_2\text{S}$ (0.84 g, 4.08 mmole) and 5 mL of dimethyl sulfide in 10 mL of dry THF at -4°C was added 1.36 mL of 3M CH_3MgBr in ether. After stirring for 10 min, the above product (1.4 g, 2.72 mmole) in 8 mL of dry THF was added. The resulting mixture was stirred at -4°C for 1 hr, and at rt for 6 hr. After cooling to -78°C , to the mixture was added 1.45

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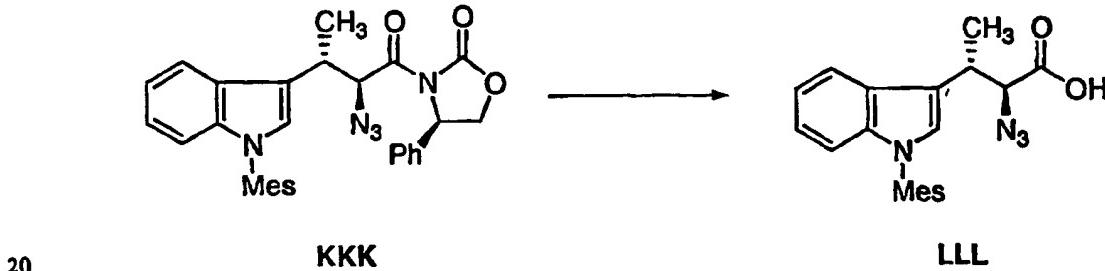
- g (8.16 mmole) of N-bromosuccinimide in 15 mL of dry THF. The mixture was stirred at -78 °C for 30 min, and at rt overnight. The mixture was poured to 100 mL of brine, and extracted with EtOAc (2x100 mL). The organic layer was dried over sodium sulfate. Filtration and concentration gave a crude product which was purified by a flash chromatography
- 5 (EtOAc/hexane, 1:4) to give the product JJJ as light brown gum in 46% yield (0.77 g). ¹H NMR (CDCl₃) δ 7.63 (d, 1H), 7.47 (s, 1H), 7.20-7.37 (m, 8H), 6.98 (s, 2H), 6.16 (d, 1H), 5.13 (dd, 1H), 4.49 (t, 1H), 4.17 (dd, 1H), 3.75 (dt, 1H), 2.54 (s, 9H), 2.31 (s, 3H), 1.59 (d, 3H).

Step 4:



- 10 The above bromide JJJ (0.72 g, 1.18 mmole) was mixed with tetra-n-butylammonium azide (1.68 g, 5.9 mmole) and sodium azide (77 mg, 1.18 mmole) in 10 mL of acetonitrile, and stirred at rt for 6 hr. The mixture poured to 100 mL of NH₄Cl aqueous solution and extracted with EtOAc (2x100 mL). The organic layer was dried over sodium sulfate. Filtration and concentration gave a crude product which was purified by a flash chromatography
- 15 (EtOAc/CH₂Cl₂/hexane, 1:2:5) to give the product KKK as light brown gum in 82% yield (0.55 g). ¹H NMR (CDCl₃) δ 7.65 (d, 1H), 7.59 (s, 1H), 7.18-7.29 (m, 8H), 6.90 (s, 2H), 5.54 (d, 1H), 5.50 (dd, 1H), 4.78 (t, 1H), 4.35 (dd, 1H), 3.62 (quintet, 1H), 2.43 (s, 9H), 2.28 (s, 3H), 1.28 (d, 3H).

Step 5:



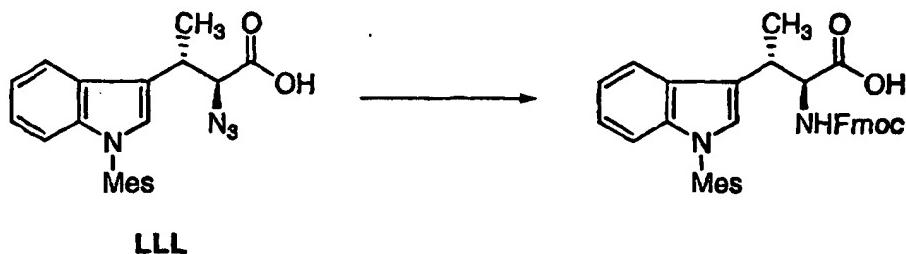
20

To a mixture of the above azide KKK (0.55 g, 0.96 mmole), water (4 mL) and THF (12 mL) at 0 °C was added 0.65 mL of 30% H₂O₂, then added 48 mg (2 equiv.) of LiOH in 1 mL water. The resulting mixture was stirred at 0 °C for 2 hr. The reaction was quenched with

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Na_2SO_3 (1 g) in 6 mL water. The mixture was stirred at rt for additional 30 min. After removal of the organic solvent, the aqueous solution was diluted with 10 mL of saturated NaHCO_3 solution and extracted with EtOAc (2x30 mL). Filtration and concentration gave a crude product which was purified by a flash chromatography (HOAc/MeOH/EtOAc, 1:10:100) to give 5 the product LLL as off-white solid in 83% yield (0.34 g). LR-Electrospray: $\text{C}_{21}\text{H}_{22}\text{N}_4\text{O}_4\text{S}$, cal: 426 observed: m/z 425 (M-H).

Step 6:



10 The above azido acid LLL (0.34 g, 0.8 mmole) was dissolved in 20 mL of methanol. To the solution was added 170 mg of 10% Pd on carbon. The resulting mixture was stirred at rt under H_2 (balloon) for 3 hr. After filtration and concentration, the crude was dissolved in a mixed solvent of THF (12 mL) and water (4 mL). To the mixture were added NaHCO_3 (254 mg, 3 mmole) and Fmoc-OSu (540 mg, 1.6 mmole). The resulting mixture was stirred at rt for 18 hr. 15 Diluted with 30 mL of saturated NH_4Cl solution and extracted with EtOAc (2x30 mL). Filtration and concentration gave a crude product which was purified by a flash chromatography (HOAc/MeOH/EtOAc, 1:10:100) to give the product 3S, 2S-Fmoc-(L)-beta methyl (Nin-Mes) tryptophan as off-white solid in 50% yield (0.25 g). LR-Electrospray: $\text{C}_{36}\text{H}_{34}\text{N}_2\text{O}_6\text{S}$, cal: 622 observed: m/z 621 (M-H).

20.

EXAMPLE 29

Preparation of Fmoc-Linker-BHA Resin

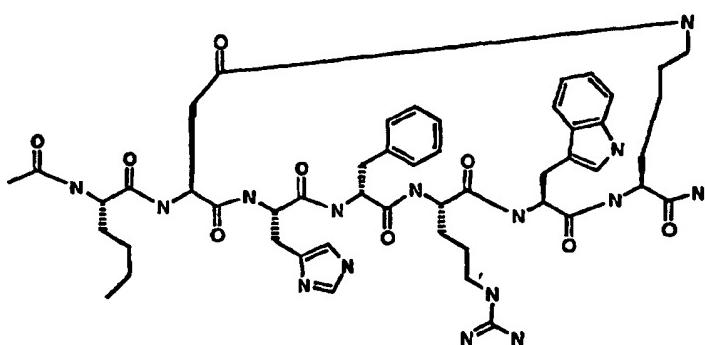
Benzhydrylamine copolystyrene-1% divinylbenzene cross-linked resin (10.0 g, 9.3 mequiv, 100-200 ASTM mesh, Advanced ChemTech) was swelled in 100 mL CH_2Cl_2 , filtered and washed 25 successively with 100 mL each of CH_2Cl_2 , 6% DIPEA/ CH_2Cl_2 (two times), CH_2Cl_2 (two times).

The resin was treated with p- [(R, S)- α -[1-(9H-fluoren-9-yl)-methoxyformamido]-2,4-

dimethoxybenzyl]-phenoxyacetic acid (Fmoc-Linker) (7.01 g, 13.0 mmole), N-hydroxybenzotriazole (2.16 g, 16.0 mmole), and diisopropylcarbodiimide (2.04 ml, 13.0 mmol) in 100 mL 25% DMF/CH₂Cl₂ for 24 hours at room temperature. The resin was filtered and washed successively with 100 ml each of CH₂Cl₂ (two times), isopropanol (two times), DMF, and CH₂Cl₂ (three times). A Kaiser ninhydrin analysis was negative. The resin was dried under vacuum to yield 16.12 g of Fmoc-Linker-BHA resin. A portion of this resin (3.5 mg) was subjected to Fmoc deprotection and quantitative UV analysis indicated a loading of 0.56 mmol/g.

10 EXAMPLE 29

Preparation of Ac-Nle-Cyclo(Asp-Lys)-Asp-His-(D)Phe-Arg-Trp-Lys-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in 15 DMF as the coupling agent and DIPEA (3 equiv.) as base. Seven coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-His (Trt) (600 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Nle (430, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 1 mL acetic anhydride in 6% 20 DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under 25 vacuum to yield 1.2 g of Ac-Heptapeptide resin. The Ac-heptapeptide resin was treated with 100

μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 250 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

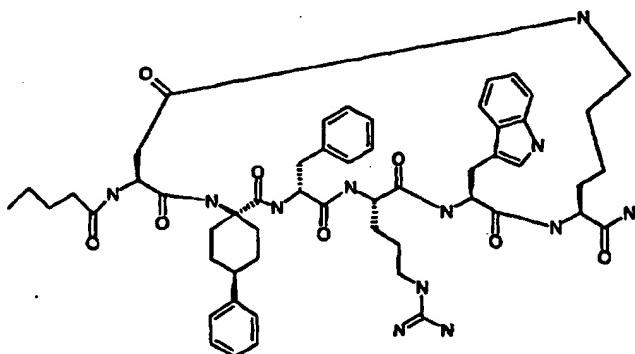
250 mg of the crude linear peptides was dissolved in 250 ml of DMF, 600 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 300 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.

10 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 60 mg (15 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₀H₆₉N₁₅O, cal: 1024 observed: m/z (1025 M+H).

EXAMPLE 31

20 Preparation of Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Arg-Trp-Lys-NH₂

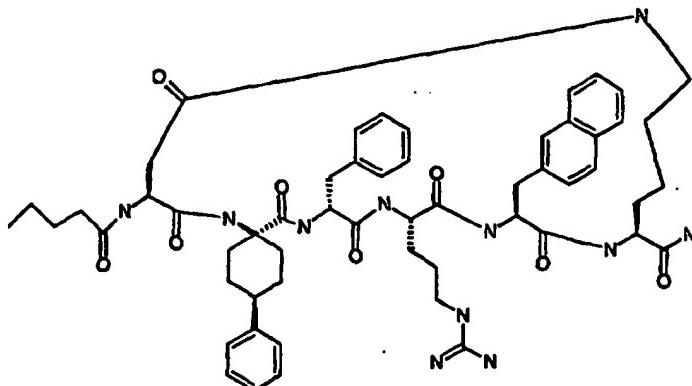


Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of

one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Asp (OBut) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.0 g of Pentyl-hexapeptide resin.

10 The Pentyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried
15 under vacuum to yield 220 mg of an off-white solid. The crude peptide was subjected to cyclization without purification. 220 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was
20 evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 53 mg (13%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray
25 C₅₄H₇₂N₁₂O₈ cal: 1017 observed: m/z (1018 M+H).

EXAMPLE 32Preparation of Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Arg-(2)Nal-Lys-NH₂

- Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(2)Nal (530 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.1 g of Pentyl-hexapeptide resin.

- The Pentyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 220 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

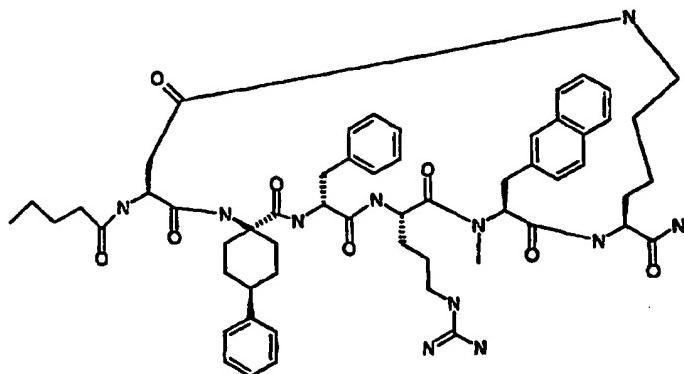
240 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 μ L of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting 5 reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 55 mg (14 %) of a 10 white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₆H₇₃N₁₁O₈ cal: 1028 observed: m/z (1029 M+H).

EXAMPLE 33

Preparation of Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Arg-N-methyl(2)Nal-Lys-NH₂

15



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Two coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), 20 Fmoc-(2)Nal (530 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol).

After Fmoc removal from 2-Nal residue, the resulting amine was converted to its 2-nitrobenzene sulfonyl derivative using 2-nitrobenzenesulfonyl chloride (5 eq., 426 mg, 1.93 mmol) and DIPEA (5 eq.) as the base in DMF. Washings were performed using DMF (6 x 30 ml) followed by CH₂Cl₂ (3 x 30 ml) and the resin was dried under vacuum. The sulfonamide

- obtained was subjected to methylation using triphenylphosphine (5 eq., 505 mg, 1.93 mmol), N, N-diethylazodicarboxylate (5 eq., 303 µL, 1.93 mmol) and methanol (10 eq. 156 µL, 3.85 mmol) in THF. Washings were performed using THF (6 x 30 ml) followed by CH₂Cl₂ (5 x 30 ml) and the resin was dried under vacuum. The 2-nitrobenzene sulfonyl group was then removed using
- 5 1,8-diazabicyclo [5.4.0] undec-7-ene (3 eq., 173 µL, 1.16 mmol), 2-mercaptopethanol (5eq. 135 µL, 1.93 mmol) in DMF. Washings were performed using DMF (3 x 30 ml), isopropanol (3 x 30 ml) followed by ethyl ether (3 x 30 ml) and the resin was dried under vacuum. The resulting N-Me-2-Nal residue was subjected to four coupling cycles, one cycle each with Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and
- 10 HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times).
- 15 The resin was dried under vacuum to yield 1.2 g of Petyl-hexapeptide resin.

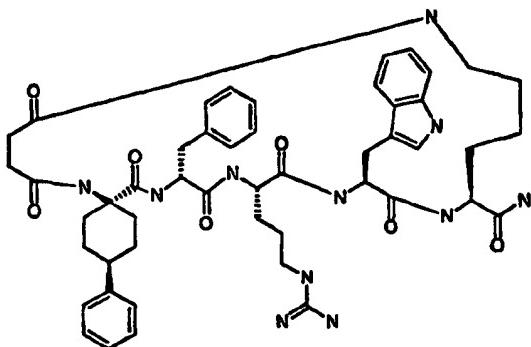
The Petyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed

20 with two or three volumes of Et₂O and re-centrifuged and the crude linear product was dried under vacuum to yield 235 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

235 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the

25 cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 mL of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 43 mg (10 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₇H₇₅N₁₁O₈ cal: 1042 observed: m/z (1043 M+H).

EXAMPLE 34**Preparation of Cyclo(succinic acid-Lys)-succinic acid-Apc-(D)Phe-Arg-Trp-Lys-NH₂**

5 Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Succinic anhydride (600 mg, 6 mmol) in DMF with 1.1 ml of DIPEA.

The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.0g
 15 Pentapeptide resin. The pentapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude 1 product was dried under
 20 vacuum to yield 220 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

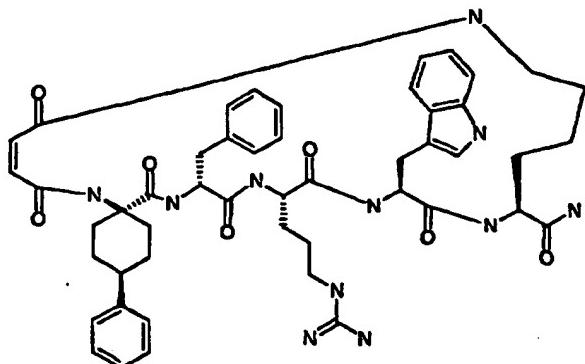
220 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.

10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 40 mg (11%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₄₉H₆₃N₁₁O₇ cal: 918 observed: m/z (919 M+H).

10 EXAMPLE 35

Preparation of Cyclo(maleic acid-Lys)-maleic acid-Apc-(D)Phe-Arg-Trp-Lys-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Maleic anhydride (600 mg, 6 mmol) in DMF with the addition of HOBT (800 mg, 6 mmol), without DIPEA. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.0 g Pentapeptide resin. The pentapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates

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were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude product was dried under vacuum to yield 230 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

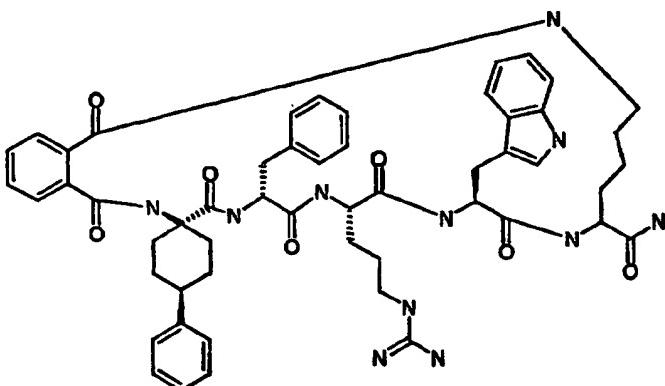
230 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 µL of N-methyl
5 morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5
10 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 38 mg (11%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₄₉H₆₁N₁₁O₇ cal: 916 observed: m/z (917 M+H).

15

EXAMPLE 36

Preparation of Cyclo(phthalic acid-Lys)-phthalic acid-Apc-(D)Phe-Arg-Trp-Lys-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid
20 phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2

mmol), Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Phthalic anhydride (660 mg, 6 mmol) in DMF with 1.1 ml of DIPEA.

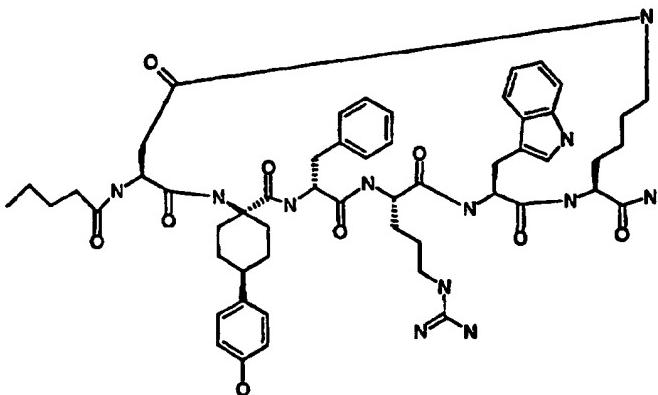
The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.0g

- 5 Pentapeptide resin. The pentapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude product was dried under
- 10 vacuum to yield 220 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

220 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.

- 15 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

- This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 35 mg (10 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₃H₆₃N₁₁O₇ cal: 966 observed: m/z (967 M+H).

EXAMPLE 37Preparation of Penta-cyclo(Asp-Lys)-Asp-4-OHApC-(D)Phe-Arg-Trp-Lys-NH₂

Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-4-OHApC (565 mg 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.1g of Pentyl-hexapeptide resin.

The Pentyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 225 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

225 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the

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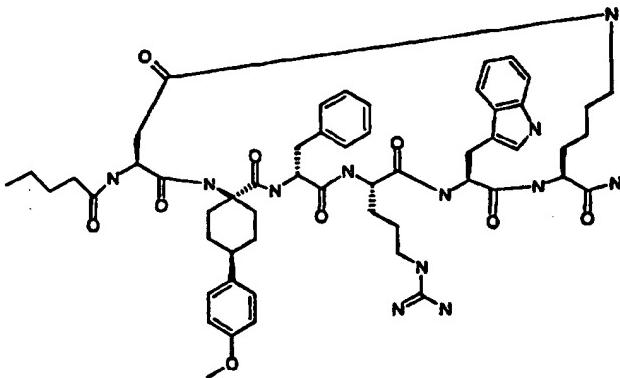
cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 55 mg (13%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₄H₇₂N₁₂O₉ cal: 1033 observed: m/z (1034 M+H).

10

EXAMPLE 38

Preparation of Penta-cyclo(Asp-Lys)-Asp-4-MeOAp-(D)Phe-Arg-Trp-Lys-NH₂



15 Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-4-MeOAp (600mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu)₂ (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively

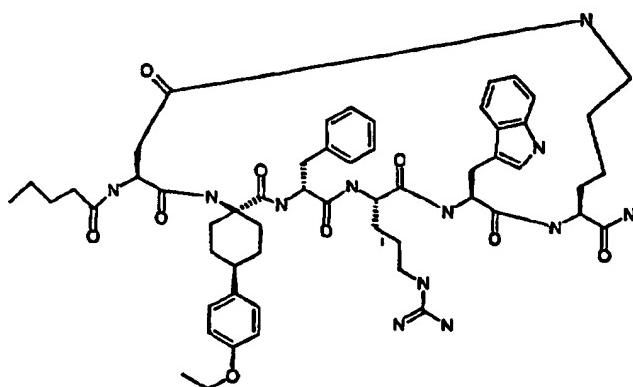
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with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.1 g of Pentyl-hexapeptide resin.

- The Pentyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min.
- 5 The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 235 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.
- 10 235 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 mL of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.
- 15 This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 49 mg (12%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray
- 20 C₅₅H₇₄N₁₂O₉, cal: 1047 observed: m/z (1048 M+H).

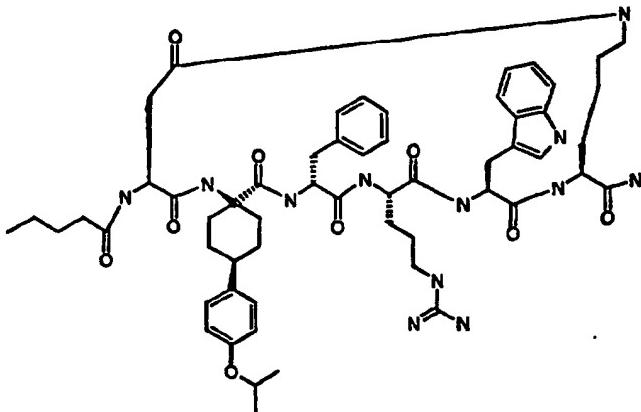
EXAMPLE 39

Preparation of Penta-cyclo(Asp-Lys)-Asp-4-EtOAp-(D)Phe-Arg-Trp-Lys-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-
5 Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-4-EtOApC (640mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu)
(500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through
10 steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.2 g of Petyl-hexapeptide resin.

The Petyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min.
15 The resin was filtered off, washed with ~2 ml TFA, and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 235 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.
20 235 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.
25 This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 60 mg (14%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray
30 C₅₆H₇₆N₁₂O₉ cal: 1061 observed: m/z (1062 M+H).

EXAMPLE 40Preparation of Penta-cyclo(Asp-Lys)-Asp-4-iPrOApC-(D)Phe-Arg-Trp-Lys-NH₂

Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-4-iPrOApC (660 mg, 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu₂) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.2 g of Pentyl-hexapeptide resin.

The Pentyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 260 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

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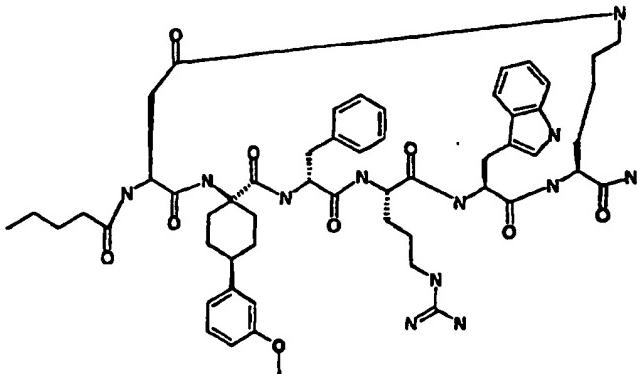
260 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 μ L of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 63 mg (15%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₇H₇₈N₁₂O₉ cal: 1075 observed: m/z (1076 M+H).

EXAMPLE 41

Preparation of Penta-cyclo(Asp-Lys)-Asp-3-MeOAp-(D)Phe-Arg-Trp-Lys-NH₂

15



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-3-MeOAp (600 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried

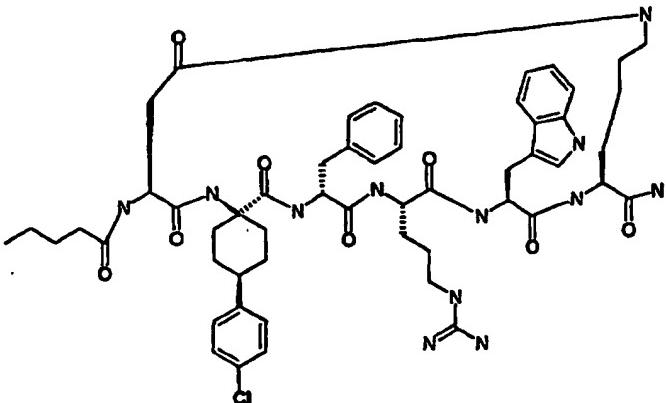
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through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.1 g of Petyl-hexapeptide resin.

- 5 The Petyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried
- 10 under vacuum to yield 235 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

- 15 235 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.
- 20 10 mL of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 49 mg (12%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₅H₇₄N₁₂O₉ cal: 1047 observed: m/z (1048 M+H).

EXAMPLE 42Preparation of Penta-cyclo(Asp-Lys)-Asp-4-ClApc-(D)Phe-Arg-Trp-Lys-NH₂

- Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-4-ClApc (560 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.0 g of Pentyl-hexapeptide resin.

The Pentyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 230 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

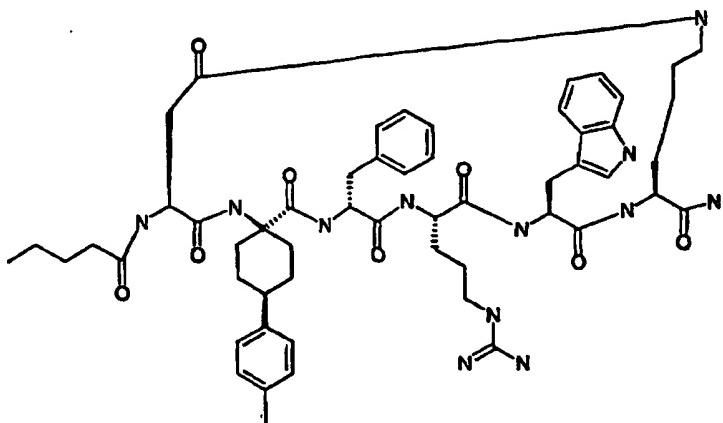
230 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.

10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

- This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min.; flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 49 mg (12 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₄H₇₁N₁₂O₈Cl ; cal: 1051 observed: m/z (1052 M+H).

10 EXAMPLE 43

Preparation of Penta-cyclo(Asp-Lys)-Asp-4-MeApc-(D)Phe-Arg-Trp-Lys-NH₂



- Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-4-MeApc (590mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.2 g of Pentyl-hexapeptide resin.

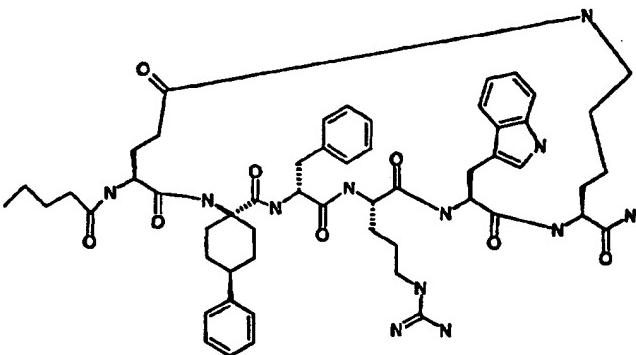
The Pentyl-hexapeptide resin was treated with 100 μ L ethanedithiol, 100 μ L dimethylsulfide, 250 μ L anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed 5 with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 240 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

240 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μ L of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the 10 cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 mL of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% 15 TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 55 mg (14 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₅H₇₄N₁₂O₈ cal: 1031 observed: m/z (1032 M+H).

20 **EXAMPLE 44**

Preparation of Penta-cyclo(Glu-Lys)-Glu-Apc-(D)Phe-Arg-Trp-Lys-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in 25 DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of

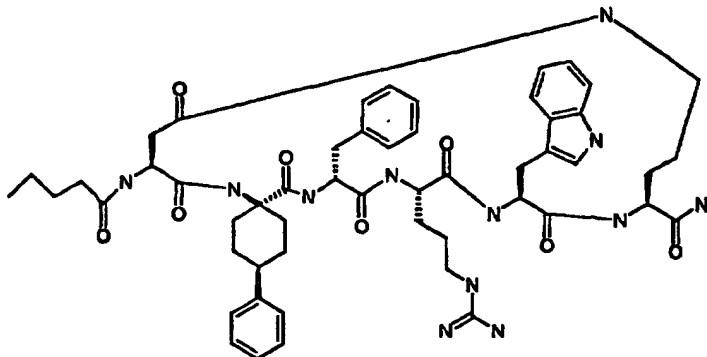
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one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Glu (OBut) (510 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.1 g of Petyl-hexapeptide resin.

10 The Petyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried
15 under vacuum to yield 255mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

255 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.
20 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 60 mg (15%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₅H₇₄N₁₂O₈ cal: 1031 observed: m/z (1032M+H).

EXAMPLE 45Preparation of Penta-cyclo(Asp-Orn)-Asp-Apc-(D)Phe-Arg-Trp-Orn-NH₂

Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Orn (Boc) (550 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBut) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.15 g of Petyl-hexapeptide resin.

The Petyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 240mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

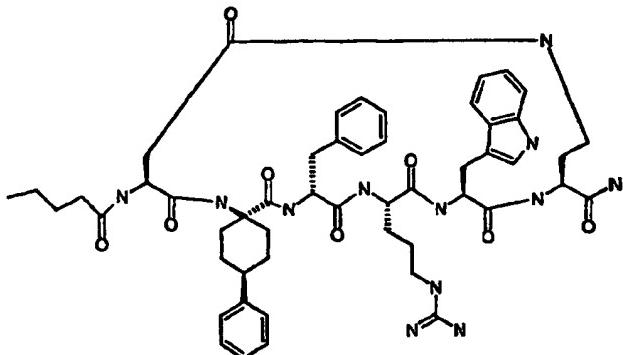
240 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.

10 ml of water was added to stop the reaction, DMF was evaporated in vacou, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 53 mg (13%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₃H₇₀N₁₂O₈ cal: 1003 observed: m/z (1004 M+H).

10 EXAMPLE 46

Preparation of Penta-cyclo(Asp-Dbr)-Asp-Apc-(D)Phe-Arg-Trp-Dbr-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in 15 DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Dbr (Boc) (540 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.10 g of Pentyl-hexapeptide resin.

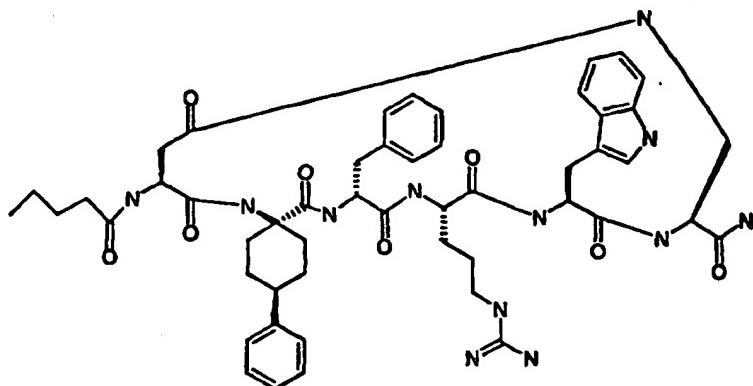
The Pentyl-hexapeptide resin was treated with 100 μ L ethanedithiol, 100 μ L dimethylsulfide, 250 μ L anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed 5 with two or three volumes of Et₂O and re-centrifuged and the crude linear product was dried under vacuum to yield 220mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

220 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 μ L of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the 10 cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% 15 TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 35 mg (9 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₂H₆₈N₁₂O₈ cal: 989 observed: m/z (990 M+H).

20 **EXAMPLE 47**

Preparation of Penta-cyclo(Asp-Dpr)-Asp-Apc-(D)Phe-Arg-Trp-Dpr-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Dpr (Boc) (530 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol),

5 Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg, 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu₂) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric

10 anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.0 g of Pentylyl-hexapeptide resin.

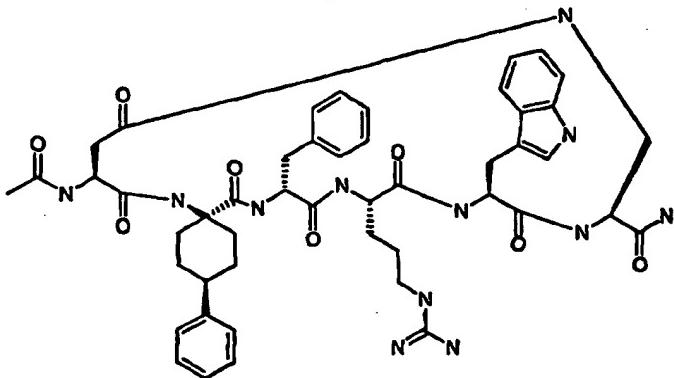
The Pentylyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min.

15 The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 200mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

20 200 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

25 This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 30 mg (8%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray

30 C₅₁H₆₆N₁₂O₈ cal: 975 observed: m/z (976 M+H).

EXAMPLE 48Preparation of Ac-cyclo(Asp-Dpr)-Asp-Apc-(D)Phe-Arg-Trp-Dpr-NH₂

Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Dpr (Boc) (530 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL acetic anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.1 g of Acetyl-hexapeptide resin.

The Acetyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 200 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

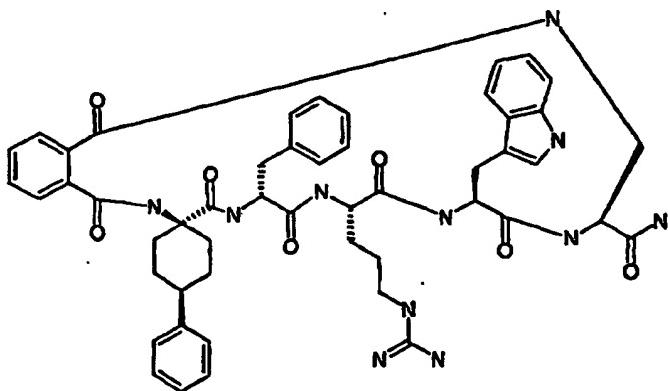
210 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.

10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% 5 TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 28 mg (8%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₄₈H₆₀N₁₂O₈ cal: m/z 933 observed: m/z (934 M+H).

10 **EXAMPLE 49**

Preparation of Cyclo(phthalic acid-Dpr)-phthalic acid-Apc-(D)Phe-Arg-Trp-Dpr-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in 15 DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Dpr (Boc) (530 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg, 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Phthalic anhydride 20 (660 mg, 6 mmol) in DMF with 1.1 ml of DIPEA. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.0 g Pentapeptide resin.

The pentapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was

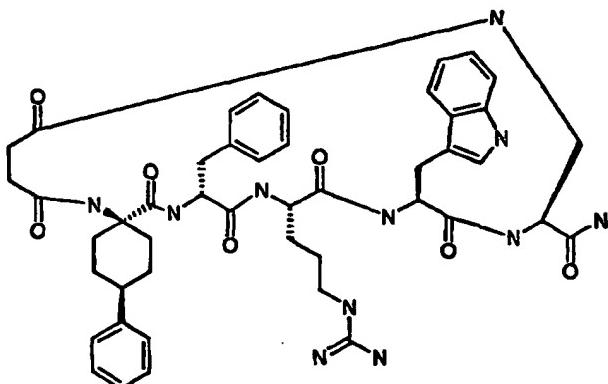
filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude product was dried under vacuum to yield 220 mg of an off-white solid. The crude peptide was subjected to cyclization without 5 purification.

220 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting 10 reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 30 mg (8 %) of a 15 white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₀H₅₇N₁₁O₇, cal: 924 observed: m/z (925 M+H).

EXAMPLE 50

Preparation of Cyclo(succinic acid-Dpr)-succinic acid-Apc-(D)Phe-Arg-Trp-Dpr-NH₂



20

Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Dpr (Boc) (530 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol),

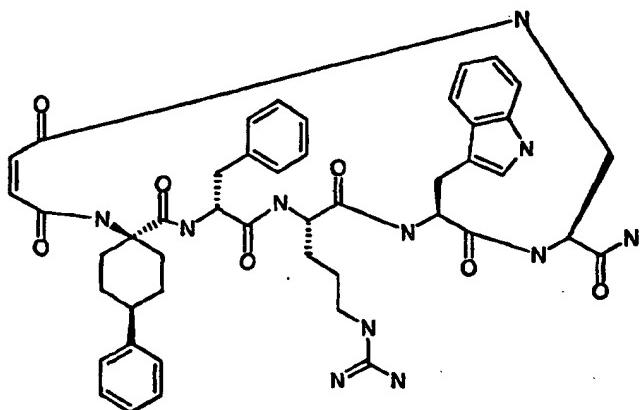
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Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Succinic anhydride (600 mg, 6 mmol) in DMF with 1.1 ml of DIPEA. The resin was filtered and washed 5 successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.0 g Pentapeptide resin.

The pentapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The 10 precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and re-centrifuged and the crude product was dried under vacuum to yield 220 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

220 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 μL of N-methyl 15 morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 20 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 31 mg (8 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₄₆H₅₇N₁₁O₇ cal: 876 observed: m/z (877 M+H).

EXAMPLE 51Preparation of Cyclo(maleic acid-Dpr)-maleic acid-Apc-(D)Phe-Arg-Trp-Dpr-NH₂

Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Dpr (Boc) (530 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Maleic anhydride (600 mg, 6 mmol) in DMF with the addition of HOBT (800 mg, 6 mmol), without DIPEA. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.0 g Pentapeptide resin.

The pentapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude product was dried under vacuum to yield 230 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

230 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.

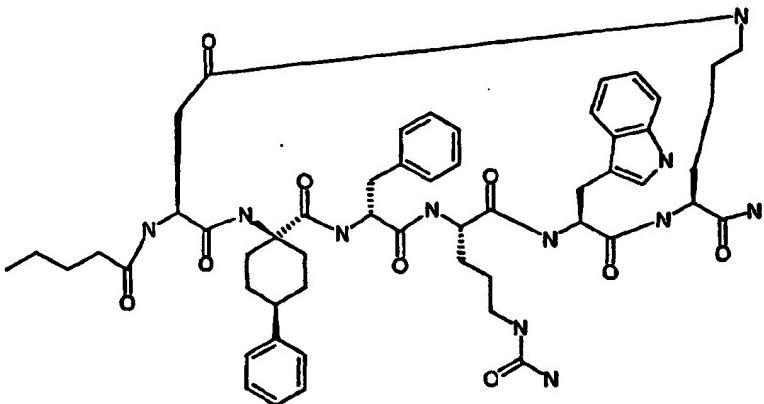
- 165 -

10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 28 mg (8 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₄₆H₅₅N₁₁O₇, cal: 874 observed: m/z (875 M+H).

10 EXAMPLE 52

Preparation of Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Cit-Trp-Lys-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Cit (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.3 g of Pentyl-hexapeptide resin.

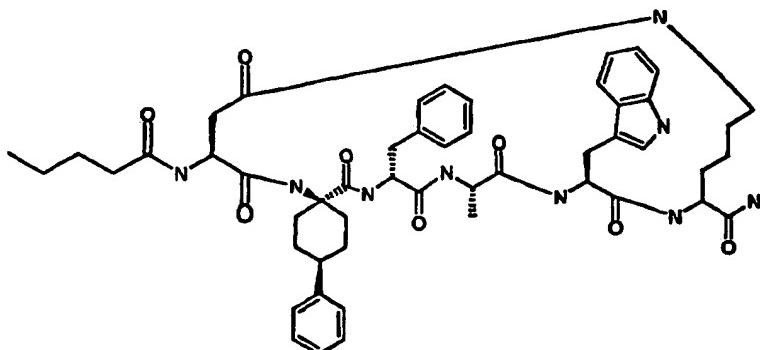
The Pentyl-hexapeptide resin was treated with 100 μ L ethanedithiol, 100 μ L dimethylsulfide, 250 μ L anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed 5 with two or three volumes of Et₂O and re-centrifuged and the crude linear product was dried under vacuum to yield 300 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

300 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μ L of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the 10 cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 mL of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% 15 TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 80 mg (20%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₄H₇₁N₁₁O₂, cal: 1018 observed: m/z (1019 M+H).

20 **EXAMPLE 53**

Preparation of Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Ala-Trp-Lys-NH₂



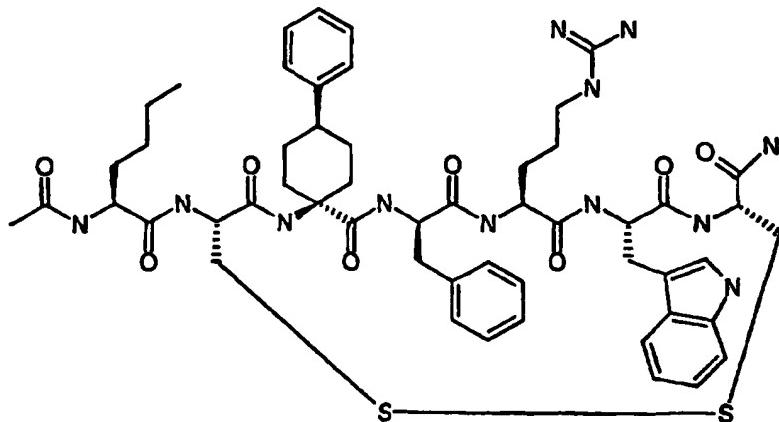
Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in

DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Ala (380 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol),
5 Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBut) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under
10 vacuum to yield 1.4 g of Petyl-hexapeptide resin.

The Petyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed
15 with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 330 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

330 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the
20 cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1%
25 TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 87 mg (20%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₁H₆₅N₉O₈ cal: 932 observed: m/z (933 M+H).

EXAMPLE 54Preparation of Ac-Nle-cyclo(Cys-Cys)-Cys-Apc-(D)Phe-Arg-Trp-Cys-NH₂

Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Seven coupling cycles were performed of one cycle each with Fmoc-Cys (Trt) (710 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Cys (Trt) (710 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Nle (430, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 1 mL acetic anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.2 g of Ac-Heptapeptide resin.

The Ac-heptapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and re-centrifuged and the crude linear product was dried under vacuum to yield 250 mg of an off-white solid.

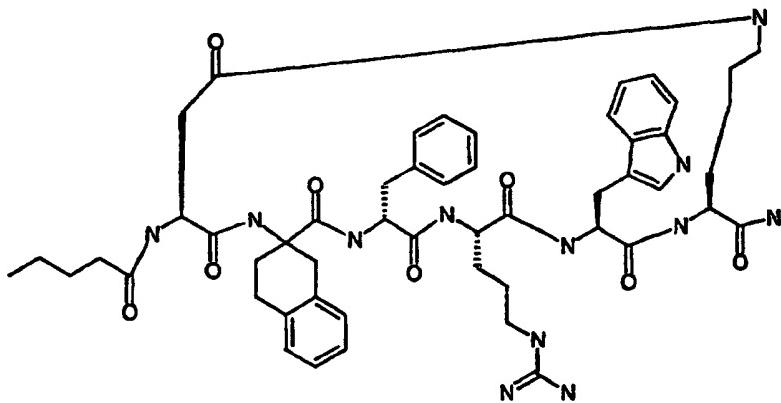
This crude linear peptide was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1%

TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 45 mg of purified linear peptide.

The purified linear peptide was dissolved in 2 ml of DMSO, diluted with 500 ml of water
 5 and the pH was adjusted to pH 8.0 with NH₄OH. O₂ was bubbled into the solution and the cyclization was monitored by HPLC. Typically the cyclization was completed within 24-48 hours. The solution was lyophilized and the material dissolved in CH₃COOH and was subjected to preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-
 10 60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 20 mg (4.7 %) of purified cyclic peptide. This compound was homogeneous by HPLC. LR-Electrospray C₅₃H₇₀N₁₂O₈S₂ cal: 1067 observed: m/z (1068 M+H).

EXAMPLE 55

15 Preparation of Penta-cyclo(Asp-Lys)-Asp-(D,L)-Atc-(D)Phe-Arg-Trp-Lys-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D,L)-Atc (510 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through

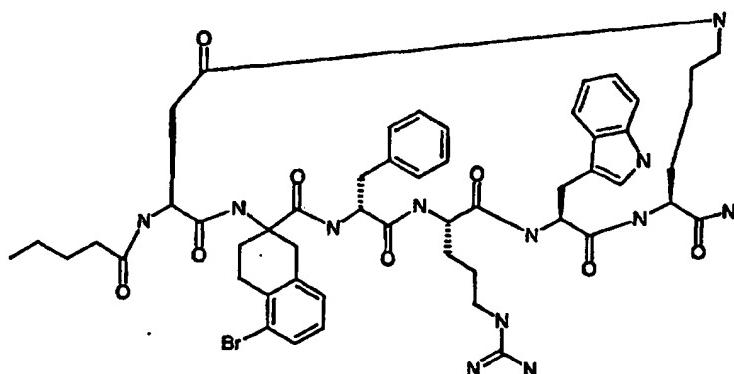
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steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.15 g of Petyl-hexapeptide resin.

- 5 The Petyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried
10 under vacuum to yield 245 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

- 250 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.
15 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

- This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by
20 analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 55 mg (14 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₂H₆₈N₁₂O₈ cal: 989 observed: m/z (990 M+H).

EXAMPLE 56Preparation of Penta-(Asp-Lys)-Asp-5-BrAtc-(D)Phe-Arg-Trp-Lys-NH₂ (peak 1)

- Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-5-Br-(D,L)Atc (620 mg, 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times).
- The resin was dried under vacuum to yield 1.1 g of Pentyl-hexapeptide resin.

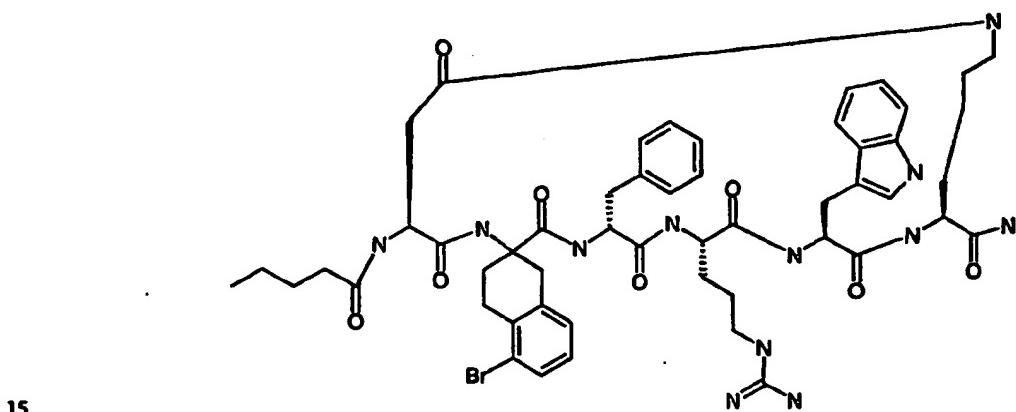
The Pentyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 240 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 240 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

240 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The first main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 26 mg (6 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₂H₆₇N₁₂O₈Br cal: 1068 observed: m/z (1069 M+H).
10

EXAMPLE 57

Preparation of Penta-(Asp-Lys)-Asp-5-BrAtc-(D)Phe-Arg-Trp-Lys-NH₂ (peak 2)



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-
 20 Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-5-Br-(D,L)Atc (620 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL

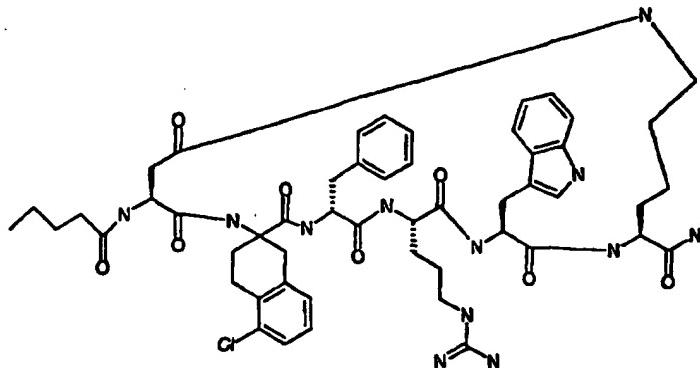
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valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.1 g of Petyl-hexapeptide resin.

- The Petyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL
- 5 dimethylsulfide, 240 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 240 mg of an off-white solid. The crude peptide was subjected to
- 10 cyclization without purification.

240 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 mL of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting 15 reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The second main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 20 mg (5 %) of 20 a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₂H₆₇N₁₂O₈Br cal: 1068 observed: m/z (1069 M+H).

EXAMPLE 58Preparation of Penta-(Asp-Lys)-Asp-5-ClAtc-(D)Phe-Arg-Trp-Lys-NH₂ (peak 1)

- Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-5-Cl(D,L)Atc (560 mg, 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times).
- The resin was dried under vacuum to yield 1.2 g of Pentyl-hexapeptide resin.

The Pentyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 250 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

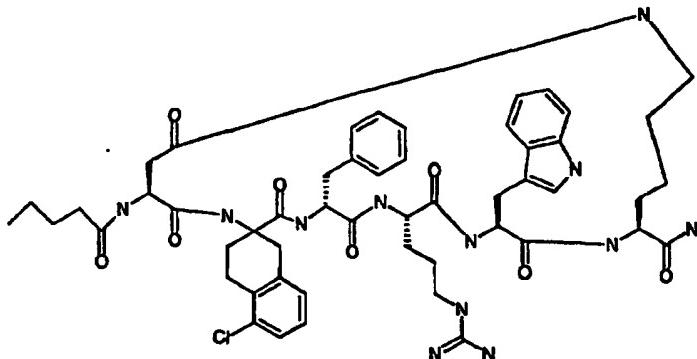
250 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.

10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydaç C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The first main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 24 mg (6%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₂H₆₇N₁₂O₈Cl cal: 1024 observed: m/z (1025 M+H).

10 EXAMPLE 59

Preparation of Penta-(Asp-Lys)-Asp-5-ClAtc-(D)Phe-Arg-Trp-Lys-NH₂ (peak 2)



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-5-Cl-(D,L)Atc (560 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.2 g of Pentyl-hexapeptide resin. The Pentyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole,

and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and re-centrifuged and the crude linear product was dried under vacuum to yield 250 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

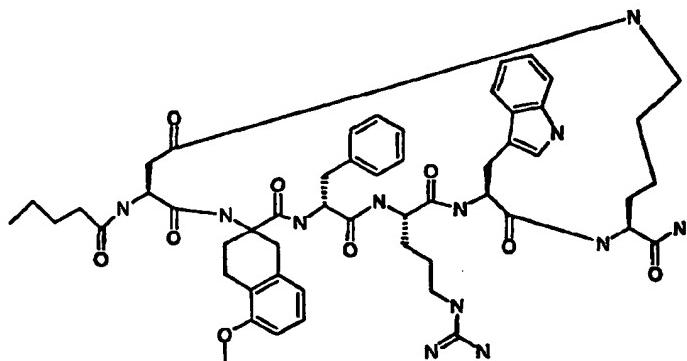
250 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 mL of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The second main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 20 mg (4%) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₂H₆₇N₁₂O₈Cl cal: 1024 observed: m/z (1025 M+H).

EXAMPLE 60

Preparation of Penta-(Asp-Lys)-Asp-5-MeO-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂

20



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-

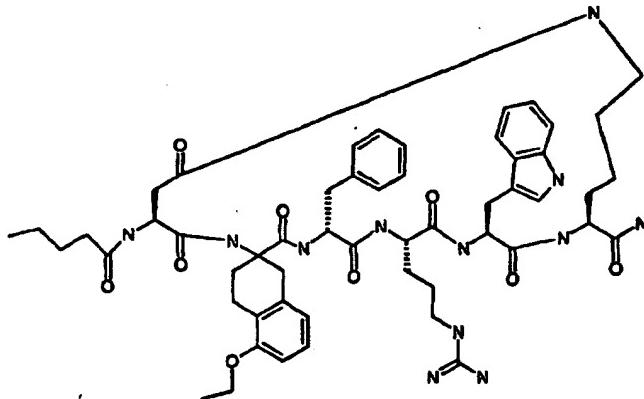
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Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-5-MeO-(D,L)Atc (600 mg, 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried
5 through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.2 g of Pentyl-hexapeptide resin.

The Pentyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL
10 dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 250 mg of an off-white solid. The crude peptide was subjected to
15 cyclization without purification.

250 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 mL of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting
20 reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 55 mg (13 %) of a
25 white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₃H₇₀N₁₂O₉ cal: 1019 observed: m/z (1020 M+H).

EXAMPLE 61Preparation of Penta-(Asp-Lys)-Asp-5-EtO-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂

- Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-5-EtO-(D,L)Atc (620 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times).
- The resin was dried under vacuum to yield 1.3 g of Pentyl-hexapeptide resin.

- The Pentyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed 20 with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 260 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

260 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the

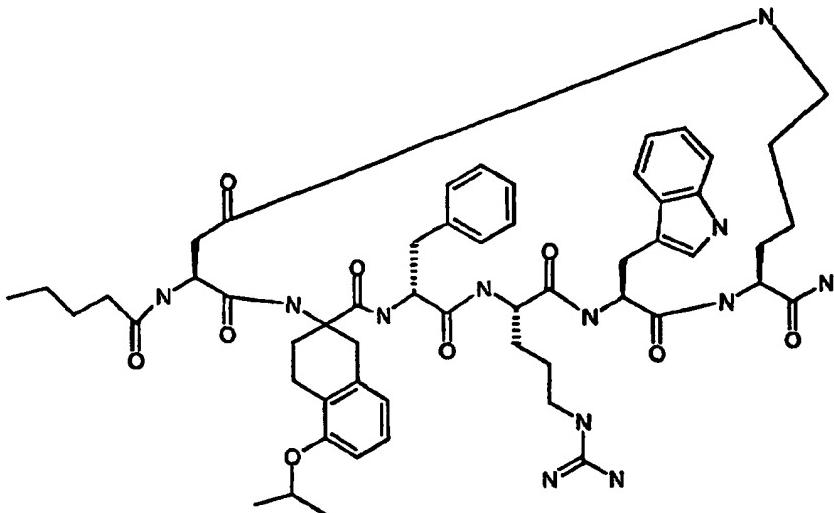
cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 58 mg (13 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₄H₇₂N₁₂O₉ cal: 1033 observed: m/z (1034 M+H).

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EXAMPLE 62

Preparation of Penta-(Asp-Lys)-Asp-5-iPrO-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid 15 phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-5-iPrO-(D,L)Atc (620 mg, 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL 20

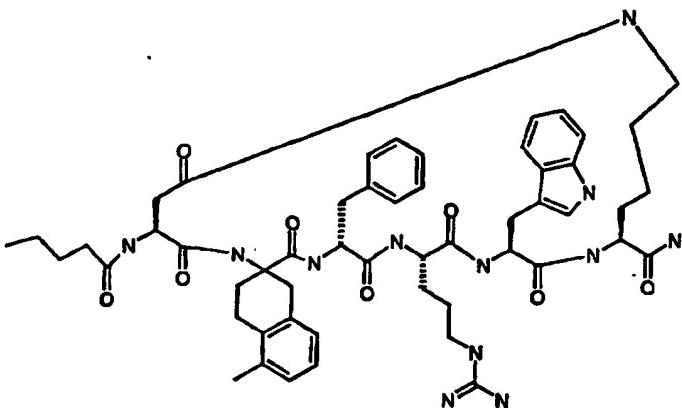
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valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.2 g of Petyl-hexapeptide resin.

- The Petyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL
5 dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 250 mg of an off-white solid. The crude peptide was subjected to
10 cyclization without purification.

- 250 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 mL of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting
15 reaction mixture was purified by HPLC.

- This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 58 mg (13 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray
20 C₅₅H₇₄N₁₂O₉ cal: 1047 observed: m/z (1048 M+H).

EXAMPLE 63Preparation of Penta-(Asp-Lys)-Asp-5-Me-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂

- Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-5-Me-(D,L)Atc (590 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times).
- The resin was dried under vacuum to yield 1.2 g of Penty-hexapeptide resin.

The Penty-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 260 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

260 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the

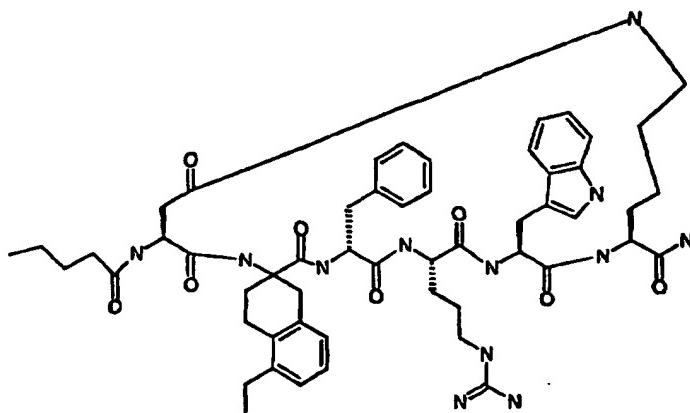
cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 62 mg (13 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₃H₇₀N₁₂O₈ cal: 1003 observed: m/z (1004 M+H).

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EXAMPLE 64

Preparation of Penta-(Asp-Lys)-Asp-5-Et-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂

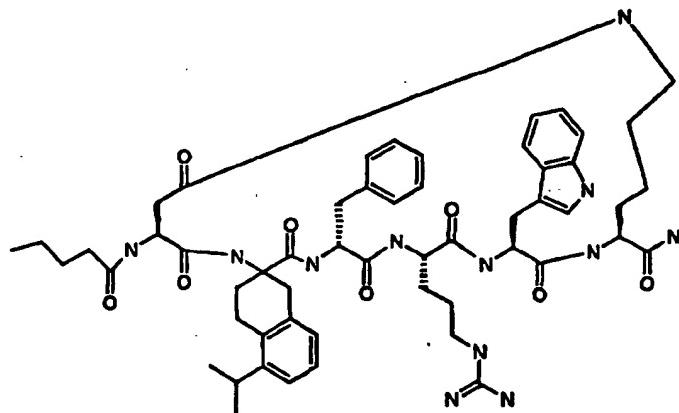


Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-5-Et-(D,L)Atc (600 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed

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successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.3 g of Pentyl-hexapeptide resin.

- The Pentyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min.
- 5 The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 245 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.
- 10 245 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 mL of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.
- 15 This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 55 mg (12 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray
- 20 C₅₄H₇₂N₁₂O₈ cal: 1017 observed: m/z (1018 M+H).

EXAMPLE 65Preparation of Penta-(Asp-Lys)-Asp-5-iPr-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂

Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-5-iPr-(D,L)Atc (600 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times).

The resin was dried under vacuum to yield 1.2 g of Penty-hexapeptide resin.

The Penty-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 245 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

245 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the

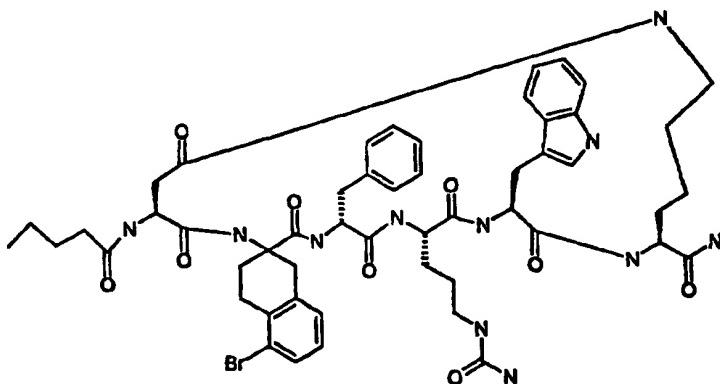
cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 54 mg (13 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₅H₇₄N₁₂O₈ cal: 1031 observed: m/z (1032 M+H).

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EXAMPLE 66

Preparation of Penta-(Asp-Lys)-Asp-5-BrAtc-(D)Phe-Cit-Trp-Lys-NH₂ (peak 1)



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid 15 phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Cit (480mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), 20 Fmoc-5-Br-(D,L)Atc (620 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was 25 dried under vacuum to yield 1.2 g of Pentyl-hexapeptide resin.

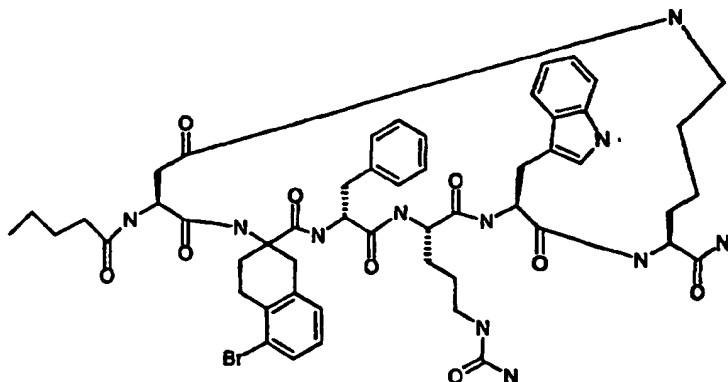
The Pentyl-hexapeptide resin was treated with 100 μ L ethanedithiol, 100 μ L dimethylsulfide, 240 μ L anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed 5 with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 260 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

240 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μ L of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the 10 cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 mL of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% 15 TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The first main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 24 mg (5.6 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₂H₆₆N₁₁O₉Br cal: 1069 observed: m/z (1070 M+H).

20 **EXAMPLE 67**

Preparation of Penta-(Asp-Lys)-Asp-5-BrAtc-(D)Phe-Cit-Trp-Lys-NH₂ (peak 2)



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in

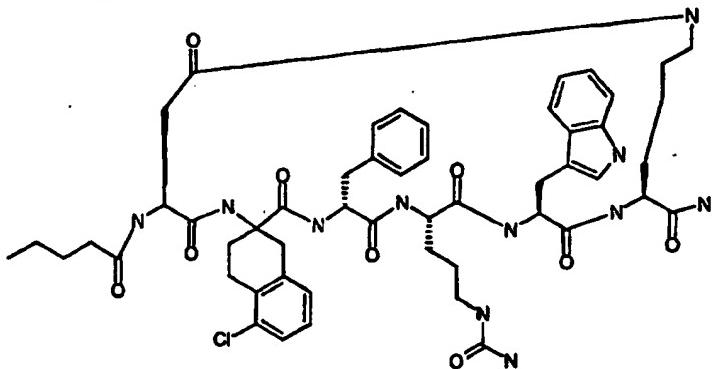
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DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Cit (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol),
5 Fmoc-5-Br-(D,L)Atc (620 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu)
(500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric
anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively
with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was
10 dried under vacuum to yield 1.1 g of Pentyl-hexapeptide resin.

The Pentyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 240 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed
15 with two or three volumes of Et₂O and re-centrifuged and the crude linear product was dried under vacuum to yield 240 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

240 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the
20 cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The second main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 22 mg (4.8 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₂H₆₆N₁₁O₉Br cal: 1069 observed: m/z (1070 M+H).

EXAMPLE 68Preparation of Penta-(Asp-Lys)-Asp-5-ClAtc-(D)Phe-Cit-Trp-Lys-NH₂ (peak 1)

Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Cit (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol),
 5 Fmoc-5-Cl-(D,L)Atc (560 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu)
 10 (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was
 15 dried under vacuum to yield 1.2 g of Pentyl-hexapeptide resin.

The Pentyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed 20 with two or three volumes of Et₂O and re-centrifuged and the crude linear product was dried under vacuum to yield 245 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

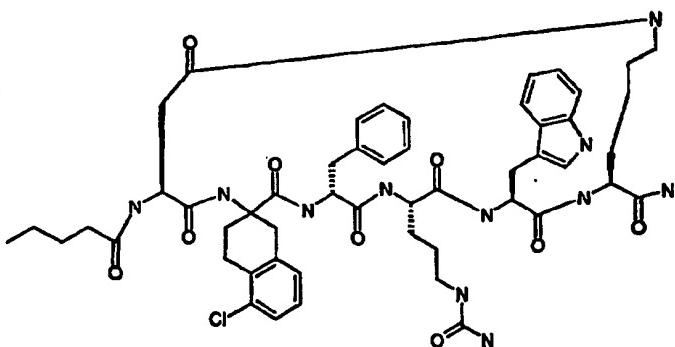
245 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the 25 cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.

10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The first main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 22 mg (5.8 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₂H₆₆N₁₁O₉Cl cal: 1024 observed: m/z (1025 M+H).

10 EXAMPLE 69

Preparation of Penta-(Asp-Lys)-Asp-5-ClAtc-(D)Phe-Cit-Trp-Lys-NH₂ (peak 2)



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in 15 DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Cit (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D,L)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-5-Cl-(D,L)Atc (560 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu^t) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.2 g of Pentyl-hexapeptide resin.

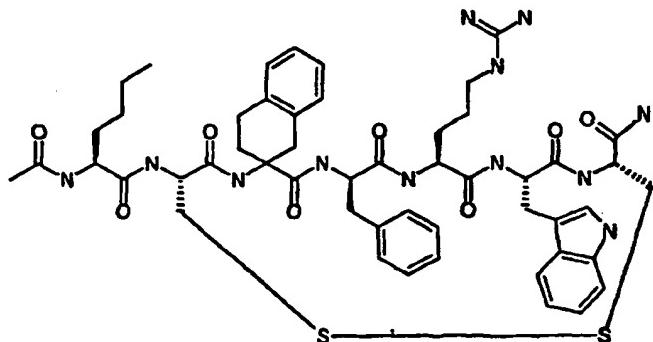
The Pentyl-hexapeptide resin was treated with 100 μ L ethanedithiol, 100 μ L dimethylsulfide, 250 μ L anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed 5 with two or three volumes of Et₂O and re-centrifuged and the crude linear product was dried under vacuum to yield 250 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

250 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μ L of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the 10 cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 mL of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% 15 TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The second main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 20 mg (5.4 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray C₅₂H₆₆N₁₁O₉Cl cal: 1024 observed: m/z (1025 M+H).

20 EXAMPLE 70

Preparation of Ac-Nle-cyclo(Cys-Cys)-Cys-(D,L)Atc-(D)Phe-Arg-Trp-Cys-NH₂



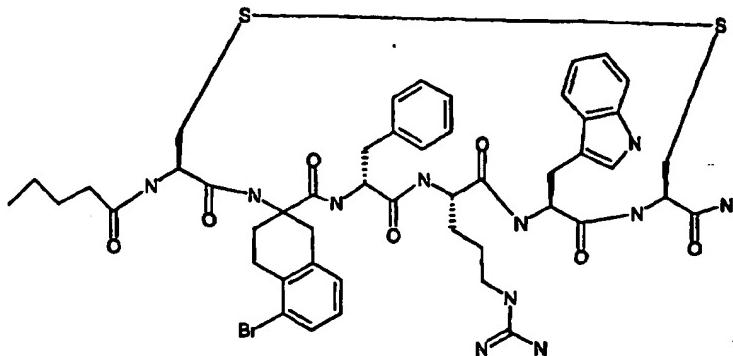
Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in

DMF as the coupling agent and DIPEA (3 equiv.) as base. Seven coupling cycles were performed of one cycle each with Fmoc-Cys (Trt) (710 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D,L)Atc (550 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Cys (Trt) (710 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Nle(430 mg ,1.2 mmol) and HBTU (452 mg,1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 1 mL acetic anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each 10 of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.0 g of Ac-Heptapeptide resin.

The Acetyl-hepta peptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA, and the filtrates precipitated in chilled ethyl 15 ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 240 mg of an off-white solid.

This crude linear peptide was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% 20 TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 55 mg of purified linear peptide.

The purified linear peptide was dissolved in 2 ml of DMSO, diluted with 500 ml of water and the pH was adjusted to pH 8.0 with NH₄OH. O₂ was bubbled into the solution and the 25 cyclization was monitored by HPLC. Typically the cyclization was completed within 24-48 hours. The solution was lyophilized and the material dissolved in CH₃COOH and was subjected to preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, 30 pooled and lyophilized to yield 20 mg (5.0 %) of purified cyclic peptide. This compound was homogeneous by HPLC. LR-Electrospray C₅₁H₆₆N₁₂O₈S₂ cal: 1039 observed: m/z (1040 M+H).

EXAMPLE 71**Preparation of Penta-cyclo(Cys-Cys)-Cys-5-Br(D,L)Atc-(D)Phe-Arg-Trp-Cys-NH₂**

Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Seven coupling cycles were performed of one cycle each with Fmoc-Cys (Trt) (710 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-5-BrAtc (620 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Cys (Trt) (710 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 1 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.1 g of Ac-Heptapeptide resin.

The pentyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA, and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and re-centrifuged and the crude linear product was dried under vacuum to yield 240 mg of an off-white solid.

This crude linear peptide was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The main peak was cut by

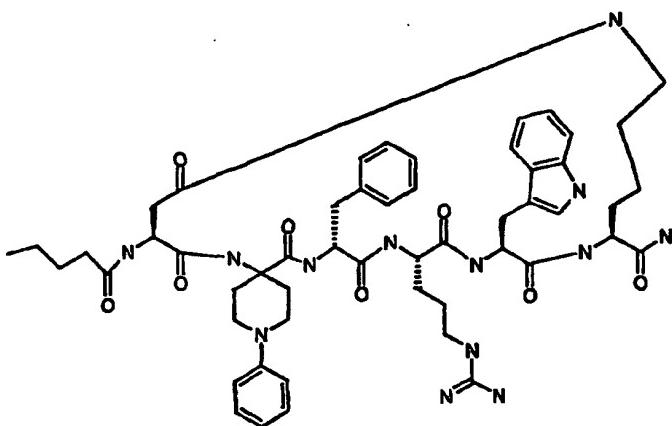
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analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 50 mg of purified linear peptide.

The purified linear peptide was dissolved in 2 ml of DMSO, diluted with 500 ml of water and the pH was adjusted to pH 8.0 with NH₄OH. O₂ was bubbled into the solution and the cyclization was monitored by HPLC. Typically the cyclization was completed within 24-48 hours. The solution was lyophilized and the material dissolved in CH₃COOH and was subjected to preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 22 mg (5.2 %) of purified cyclic peptide. This compound was homogeneous by HPLC. LR-Electrospray C₄₈H₆₀N₁₁O₇S₂ Br cal: 1047 observed: m/z (1048 M+H).

EXAMPLE 72

15 Preparation of Penta-cyclo(Asp-Lys)-Asp-Appc-(D)Phe-Arg-Trp-Lys-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of 20 one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Appc (550 mg, 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 -

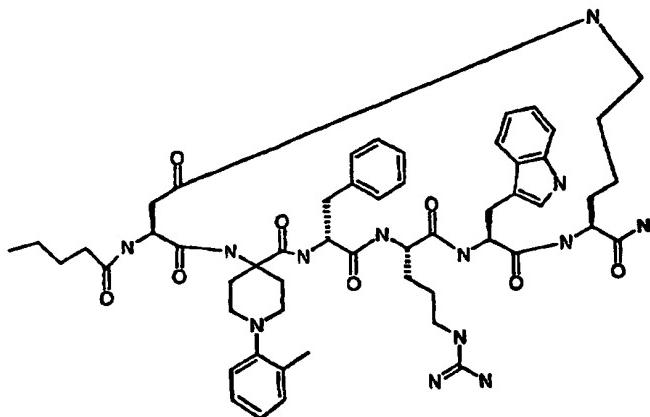
- 194 -

5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.2 g of Petyl-hexapeptide resin.

- 5 The Petyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and re-centrifuged and the crude linear product was dried
10 under vacuum to yield 245 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

- 245 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.
15 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

- This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 57 mg (14 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray:
20 C₅₃H₇₁N₁₃O₈ cal: 1018 observed: m/z (1019 M+H).

EXAMPLE 73Preparation of Penta-cyclo(Asp-Lys)-Asp-2-MeAppc-(D)Phe-Arg-Trp-Lys-NH₂

Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-2-MeAppc (570 mg, 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu)₂ (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.2 g of Pentyl-hexapeptide resin.

The Pentyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and re-centrifuged and the crude linear product was dried under vacuum to yield 250 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

250 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the

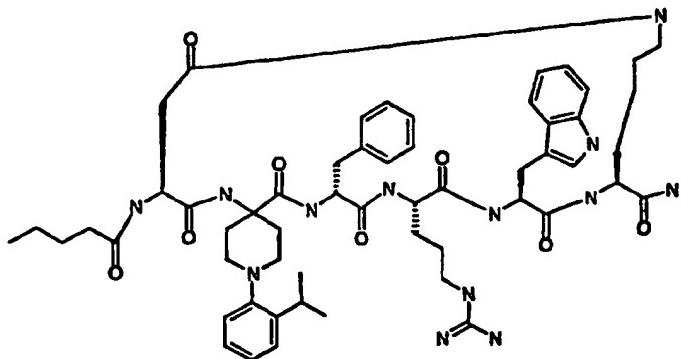
cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 61 mg (15 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray: C₅₄H₇₃N₁₃O₈ cal: 1032 observed: m/z (1033M+H).

10

EXAMPLE 74

Preparation of Penta-cyclo(Asp-Lys)-Asp-2-iPrAppc-(D)Phe-Arg-Trp-Lys-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-2-iPrAppc (600 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.2 g of Pentyl-hexapeptide resin.

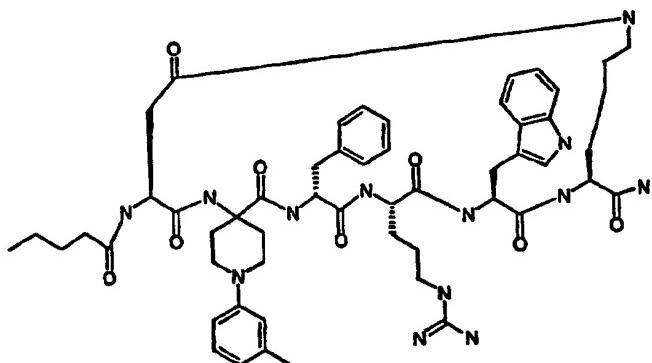
The Pentyl-hexapeptide resin was treated with 100 μ L ethanedithiol, 100 μ L dimethylsulfide, 250 μ L anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed 5 with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 245 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

245 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μ L of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the 10 cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 mL of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% 15 TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 52 mg (14 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray: C₅₆H₇₇N₁₃O₈ cal: 1060 observed: m/z (1061 M+H).

20 EXAMPLE 75

Preparation of Penta-cyclo(Asp-Lys)-Asp-3-MeAppc-(D)Phe-Arg-Trp-Lys-NH₂



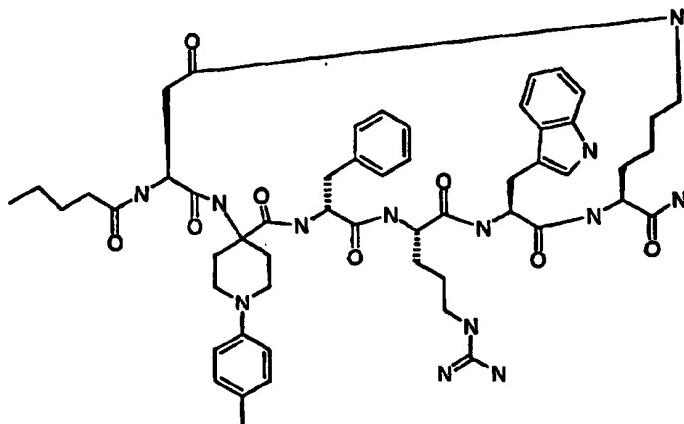
Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in

DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-3-MeAppc (570 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.2 g of Petyl-hexapeptide resin.

The Petyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 248 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

248 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 55 mg (14 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray: C₅₄H₇₃N₁₃O₈ cal: 1032 observed: m/z (1033 M+H).

EXAMPLE 6Preparation of Penta-cyclo(Asp-Lys)-Asp-4-MeAppc-(D)Phe-Arg-Trp-Lys-NH₂

Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-4-MeAppc (570 mg, 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.2 g of Pentyl-hexapeptide resin.

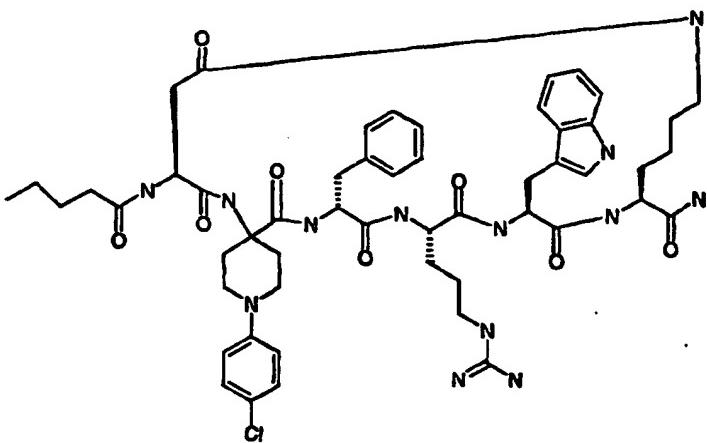
The Pentyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 254 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

254 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 μ L of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting 5 reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 57 mg (14 %) of a 10 white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray: C₅₄H₇₃N₁₃O₈ cal: 1032 observed: m/z (1033 M+H).

EXAMPLE 77

Preparation of Penta-cyclo(Asp-Lys)-Asp-4-ClAppc-(D)Phe-Arg-Trp-Lys-NH₂



15

Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-20 Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-4-ClAppc (580 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu₂) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through

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steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.2 g of Penty1-hexapeptide resin.

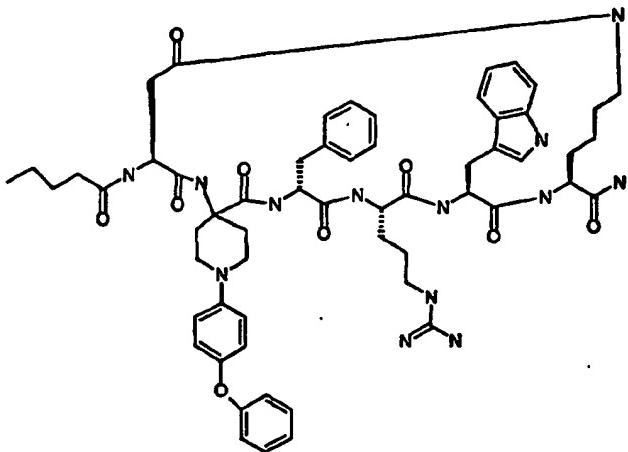
- 5 The Penty1-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL dimethylsulfide, 250 µL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried
10 under vacuum to yield 250 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

- 250 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 µL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.
15 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

- This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 55 mg (14 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray:
20 C₅₃H₇₀N₁₃O₈Cl cal: 1032 observed: m/z (1033 M+H).

EXAMPLE 78

Preparation of Penta-cyclo(Asp-Lys)-Asp-4-PhOAppc-(D)Phe-Arg-Trp-Lys-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid

- 5 phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-4-PhOAppc (650 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times).
- 10 15 The resin was dried under vacuum to yield 1.2 g of Penty-hexapeptide resin.

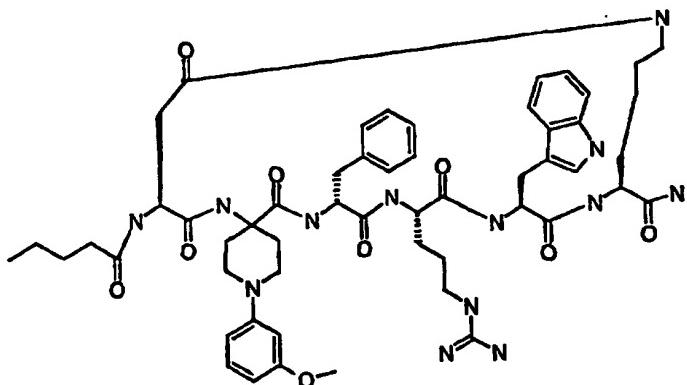
- The Penty-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 270 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

270 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 μ L of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting 5 reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 58 mg (13 %) of a 10 white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray: C₅₉H₇₅N₁₃O₉ cal: 1110 observed: m/z (1111 M+H).

EXAMPLE 79

Preparation of Penta- (Asp-Lys)-Asp-3-MeO-Appc-(D)Phe-Arg-Trp-Lys- NH₂



15

Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc- 20 Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-3-MeOAppc (580 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL

valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.2 g of Pentyl-hexapeptide resin.

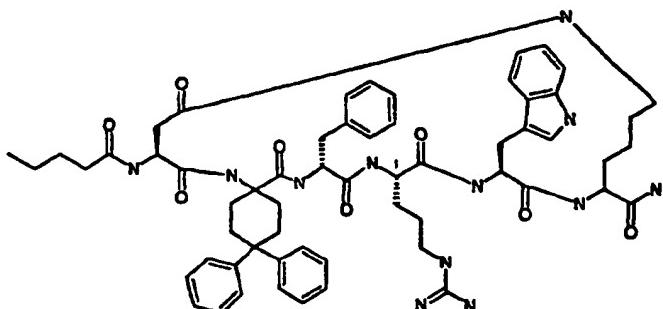
- The Pentyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL 5 dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and re-centrifuged and the crude linear product was dried under vacuum to yield 250 mg of an off-white solid. The crude peptide was subjected to 10 cyclization without purification.

- 250 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 mL of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting 15 reaction mixture was purified by HPLC.

- This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 54 mg (13 %) of a 20 white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray: C₅₄H₇₃N₁₃O₉ cal: 1048 observed: m/z (1049 M+H).

EXAMPLE 80

Preparation of Penta-cyclo(Asp-Lys)-Asp-4-Adpc-(D)Phe-Arg-Trp-Lys-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-
5 Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol)
and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2
mmol), Fmoc-Adpc (620 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500
mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 -
10 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in
each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under
vacuum to yield 1.1 g of Petyl-hexapeptide resin.

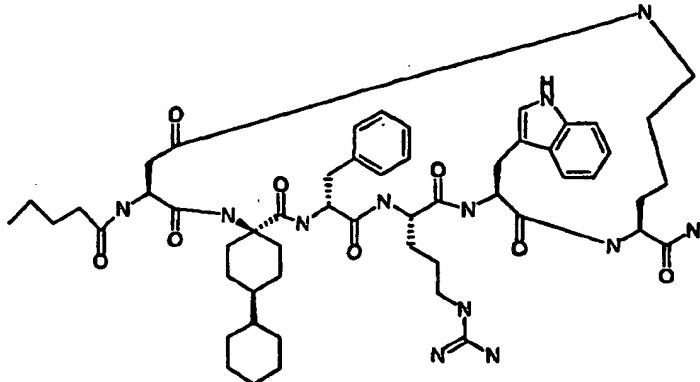
The Petyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL
dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min.
15 The resin was filtered off, washed with ~2 ml TFA and the filtrates precipitated in chilled ethyl
ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed
with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried
under vacuum to yield 242 mg of an off-white solid. The crude peptide was subjected to
cyclization without purification.

20 242 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 μL of N-methyl
morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the
cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.
10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting
reaction mixture was purified by HPLC.

25 This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5
x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1%
TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by
analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 48 mg (11%) of a
white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray:
30 C₆₀H₇₆N₁₂O₈ cal: 1093 observed: m/z (1094 M+H).

EXAMPLE 81

Preparation of Penta-cyclo(Asp-Lys)-Asp-Achc-(D)Phe-Arg-Trp-Lys-NH₂



Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Achc (560 mg 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 1.1 g of Petyl-hexapeptide resin.

The Petyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min. The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and re-centrifuged and the crude linear product was dried under vacuum to yield 250 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

250 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the

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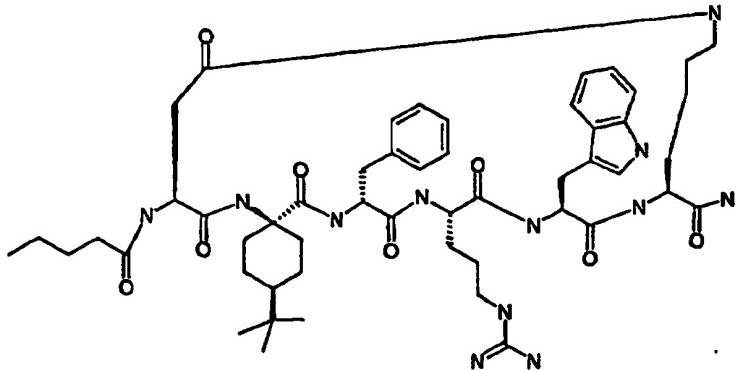
cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting reaction mixture was purified by HPLC.

This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 52 mg (13 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray: C₅₄H₇₈N₁₂O₈ cal: 1023 observed: m/z (1024 M+H).

10

EXAMPLE 82

Preparation of Penta-cyclo(Asp-Lys)-Asp-Abc-(D) Phe-Arg-Trp-Lys-NH₂



15 Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Trp (520 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg (Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Abc (530 mg, 1.2 mmol) and HBTU (452 mg, 0.6 mmol), Fmoc-Asp (OBu_t) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was carried through steps 1 - 20 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed successively with 50 ml

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each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times). The resin was dried under vacuum to yield 13 g of Pentyl-hexapeptide resin.

The Pentyl-hexapeptide resin was treated with 100 μL ethanedithiol, 100 μL dimethylsulfide, 250 μL anisole, and 10 mL trifluoroacetic acid at room temperature for 180 min.

- 5 The resin was filtered off, washed with ~2 mL TFA and the filtrates precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the crude linear product was dried under vacuum to yield 255 mg of an off-white solid. The crude peptide was subjected to cyclization without purification.

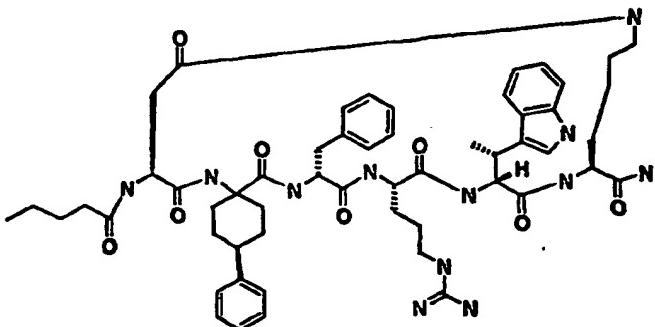
- 10 255 mg of the crude linear peptides was dissolved in 220 mL of DMF, 500 μL of N-methyl morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours. 10 mL of water was added to stop the reaction, DMF was evaporated in vacuum and the resulting reaction mixture was purified by HPLC.

- 15 This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5 x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1% TFA/CH₃CN) in 90 min., flow rate 8 mL/min, detection 280 nm. The main peak was cut by analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 58 mg (14 %) of a white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray

- 20 C₅₂H₇₆N₁₂O₈ cal: 997 observed: m/z (998 M+H).

EXAMPLE 83

Preparation of Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Arg-(2S,3S) beta methyl-Trp-Lys-NH₂



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Fmoc-Linker-BHA resin (720 mg, 0.4 mmol) from Example 29 were subjected to solid phase synthesis using Protocol 1 described above. All couplings were performed using HBTU in DMF as the coupling agent and DIPEA (3 equiv.) as base. Six coupling cycles were performed of one cycle each with Fmoc-Lys (Boc) (565 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-
5 (2S,3S) beta methyl (nMes)Trp (616 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-Arg
(Pmc) (800 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol), Fmoc-(D)Phe (480 mg, 1.2 mmol)
and HBTU (452 mg, 1.2 mmol), Fmoc-Apc (550 mg 1.2 mmol) and HBTU (452 mg, 1.2 mmol),
Fmoc-Asp (OBut) (500 mg, 1.2 mmol) and HBTU (452 mg, 1.2 mmol). The peptide resin was
carried through steps 1 - 5 of protocol 1, washed with CH₂Cl₂ (three times) and treated with 2
10 mL valeric anhydride in 6% DIPEA/CH₂Cl₂ for 30 minutes. The resin was filtered and washed
successively with 50 ml each of CH₂Cl₂ (two times), isopropanol, and CH₂Cl₂ (three times).
The resin was dried under vacuum to yield 1.0 g of Petyl-hexapeptide resin.

The Petyl-hexapeptide resin was treated with 100 µL ethanedithiol, 100 µL
dimethylsulfide, 250 µL anisole, and 10 mL HF at 0C for 60 min. HF was evaporated, and the
15 resin was washed with ethylacetate filtered off, washed with ~5 ml TFA and the filtrates
precipitated in chilled ethyl ether. The precipitates were centrifuged and the ether layer
decanted. The residue was washed with two or three volumes of Et₂O and recentrifuged and the
crude linear product was dried under vacuum to yield 180 mg of an off-white solid. The crude
peptide was subjected to cyclization without purification.

20 180 mg of the crude linear peptides was dissolved in 220 ml of DMF, 500 µL of N-methyl
morpholine was added to have an apparent pH of 8.0. 280 mg of BOP was added and the
cyclization was monitored by HPLC. Typically the cyclization was completed within 18-24 hours.
10 ml of water was added to stop the reaction, DMF was evaporated in vacuum, and the resulting
reaction mixture was purified by HPLC.

25 This crude cyclic material was purified by preparative HPLC on a Vydac C18-column (2.5
x 20 cm) and eluted with a linear gradient of 20-60% B (buffer A: 0.1% TFA/H₂O, buffer B: 0.1%
TFA/CH₃CN) in 90 min., flow rate 8 ml/min, detection 280 nm. The main peak was cut by
analytical HPLC analysis of collected fractions, pooled and lyophilized to yield 40 mg (10%) of a
white, amorphous powder. This compound was homogeneous by HPLC. LR-Electrospray
30 C₅₅H₇₄N₁₂O₈ cal: 1031 observed: m/z (1032 M+H).

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BIOLOGICAL ACTIVITY EXAMPLE:

Example A: Agonist Assay

5 Method

Description: HEK 293 cells transfected with either the MC-4 receptor or MC-1 receptor were grown in 96 well plates. The cells were stimulated with either 100nM NDP- α MSH or screening compounds. Cyclic AMP was extracted from the cells and concentrations were determined using a Biotrak-cAMP SPA assay. Agonists were identified as those compounds causing an increase in
10 cAMP.

Cell Culture: HEK 293 cells transfected with either the MC-4 receptor or MC-1 receptor were cultured in 75cm² flasks in D-MEM supplemented with 10% FCS and 500 μ g/ml G418. Cells were trypsinized and split 1:3 into 96 well flat-bottom tissue culture treated plates. Cells were
15 stimulated at confluence (day 2-4).

cAMP Response: Compounds serially diluted in 100% DMSO were further diluted 1:200 (2.5 μ l compound dilution + 500 μ l media) in D-MEM containing 10%FBS and 0.1mM IBMX. For unstimulated cells, 2.5 μ l of DMSO was added to 500 μ l of media. For NDP- α MSH stimulated cells, 2.5 μ l of 20 μ M NDP- α MSH in 100% DMSO was added to 500 μ l of media (final conc.
20 100nM). Final concentration of DMSO in all wells was 0.5%.

Note: Each sample was run in duplicate on separate plates

Culture medium was removed from confluent 96 well culture plates and replaced with 200 μ l of
25 above dilutions into the appropriate wells . The plates were incubated for 1hr at RT. The media was removed, and the plates were washed 1x with 200 μ l well of PBS. CAMP was extracted by the addition of 60 μ l 70% ethanol (stored in the refrigerator). After a 30min extraction period, 10 μ l ethanol extract was transferred to the cAMP assay plate or samples were stored at -20°C until the cAMP assay was performed.

cAMP Assay: The extracted samples and all reagents included in the kit were brought to room temperature. To a 96 well OptiPlate, 10 μ l ethanol extract, 40 μ l assay buffer, 50 μ l [125I]cAMP, 50 μ l antiserum and 50 μ l SPA beads were added. The total well volume after addition was 200 μ l. The plates were sealed and incubated for 15-20 hr at room temperature. [125I]cAMP binding to the SPA beads was determined by counting each plate for 2 minutes on a Packard TopCount™.

Note: Each plate contained samples of controls for unstimulated cells and NDP- α MSH for stimulated cells.

10 The results are shown in the following table I.

Table I:

Example	Compounds	MC-1 (EC50 nM)	MC-4 (EC50 nM)
30	Ac-Nle-Cyclo(D-K)-Asp-His-(D)Phe-Arg-Trp-Lys-NH ₂	0.6	0.6
31	Penta-Cyclo(D-K)-Asp-Apc-(D)Phe-Arg-Trp-Lys-NH ₂	654	9.2
32	Penta-Cyclo(D-K)-Asp-Apc-(D)Phe-Arg-(2)Nal-Lys-NH ₂	310	7
34	Cyclo(succ-K)-succinic-Asp-Apc-(D)Phe-Arg-Trp-Lys-NH ₂	985	6.4
35	Cyclo(maleic-K)-maleic-Asp-Apc-(D)Phe-Arg-Trp-Lys-NH ₂		2.9
36	Cyclo(phthalic-K)-phthalic-Asp-Apc-(D)Phe-Arg-Trp-Lys-NH ₂	2150	3.1
38	Penta-Cyclo(D-K)-Asp-4-MeO Apc-(D)Phe-Arg-Trp-Lys-NH ₂	999,999	8.4
40	Penta-Cyclo(D-K)-Asp-4-iPrO Apc-(D)Phe-Arg-Trp-Lys-NH ₂	50%@50 μ M	6.1
41	Penta-Cyclo(D-K)-Asp-3-MeO Apc-(D)Phe-Arg-Trp-	76	1.5

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	Lys-NH ₂		
42	Penta-Cyclo(D-K)-Asp4-ClApc-(D)Phe-Arg-Trp-Lys-NH ₂	796	2.2
43	Penta-Cyclo(D-K-Asp-4-MeApc-(D)Phe-Arg-Trp-Lys-NH ₂	719	2.2
44	Penta-Cyclo(D-K)-Glu-Apc-(D)Phe-Arg-Trp-Lys-NH ₂	168	1.3
45	Penta-Cyclo(D-K)-Asp-Apc-(D)Phe-Arg-Trp-Orn-NH ₂	154	1.1
46	Penta-Cyclo(D-K)-Asp-Apc-(D)Phe-Arg-Trp-Dbr-NH ₂	848	2.5
47	Penta-Cyclo(D-K)-Asp-Apc-(D)Phe-Arg-Trp-Dpr-NH ₂	1180	6.9
48	Ac-Cyclo(D-K)-Asp-Apc-(D)Phe-Arg-Trp-Dpr-NH ₂	2969	7.4
59	Penta-Cyclo(D-K)-Asp-5ClAtc-(D)Phe-Arg-Trp-Lys-NH ₂	2130 (PA)	4
60	Penta-Cyclo(D-K)-As-5-MeO(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH ₂		4.8
62	Penta-Cyclo(D-K)-As-5-iPrO(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH ₂		7.3
64	Penta-Cyclo(D-K)-As-5-Et(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH ₂	422	8.4
65	Penta-Cyclo(D-K)-As-5-iPr(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH ₂		6.7
72	Penta-Cyclo(D-K)-Asp-Apc-(D)Phe-Arg-Trp-Lys-NH ₂	268	3.6
73	Penta-Cyclo(D-K)-Asp-2-MeAppc-(D)Phe-Arg-Trp-Lys-NH ₂	213	3.2

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74	Penta-Cyclo(D-K)-Asp-2-iPrAppc-(D)Phe-Arg-Trp-Lys-NH ₂		10
75	Penta-Cyclo(D-K)-Asp-3-MeAppc-(D)Phe-Arg-Trp-Lys-NH ₂	221	2.6
76	Penta-Cyclo(D-K)-Asp-4-MeAppc-(D)Phe-Arg-Trp-Lys-NH ₂	770	6.3
77	Penta-Cyclo(D-K)-Asp-4-ClAppc-(D)Phe-Arg-Trp-Lys-NH ₂	330	9.1
78	Penta-Cyclo(D-K)-Asp-4-PhOAppc-(D)Phe-Arg-Trp-Lys-NH ₂		6.7
79	Penta-Cyclo(D-K)-Asp-3-MeOAppc-(D)Phe-Arg-Trp-Lys-NH ₂		4.7
81	Penta-Cyclo(D-K)-Asp-Achc-(D)Phe-Arg-Trp-Lys-NH ₂	160	6.3
82	Penta-Cyclo(D-K)-Asp-Abc-(D)Phe-Arg-Trp-Lys-NH ₂	941	4.2
83	Penta-Cyclo(D-K)-Asp-Ape-(D)Phe-Arg-Trp-Lys-NH ₂	510	1.4

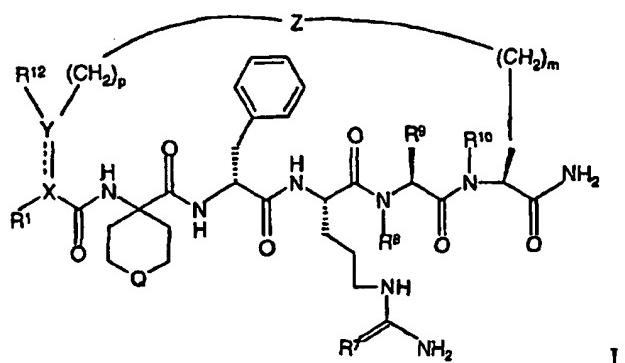
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Example B: Injection solutions may have the following composition:

Compound of formula I	3.0 mg
Gelatine	150.0 mg
Phenol	4.7 mg
Water for injection solutions	ad 1.0 ml

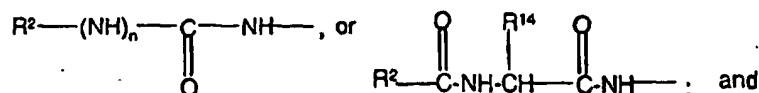
CLAIMS

1. A compound of the formula:



5 wherein

R¹ and R¹² together with X and Y form a phenyl ring and X is C and Y is C, or
R¹ is hydrogen,



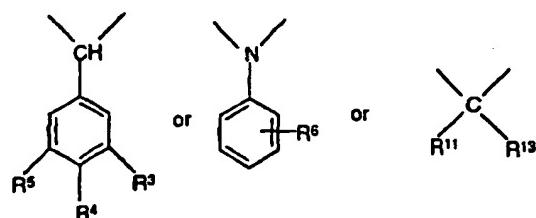
10 R¹² is hydrogen, with either X and Y being each C and the bond between X and Y being a double bond, or with X and Y being each CH and the bond between X and Y being a single bond;

15 R² is alkyl having from 1 to 5 carbon atoms, alkenyl having from 2 to 5 carbon atoms, or alkynyl having from 2 to 5 carbon atoms;

R¹⁴ is alkyl having from 1 to 5 carbon atoms;

n is 0 or 1; and

Q is



wherein R³, R⁴ and R⁵ are independently hydrogen, halo, alkyl having from 1 to 4 carbon atoms, hydroxy or alkoxy having from 1 to 4 carbon atoms, wherein when R⁴ is not hydrogen, R³ and R⁵ are both hydrogen; and

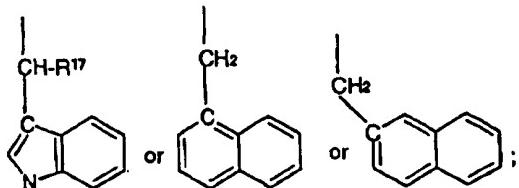
R⁶ is hydrogen, alkyl having from 1 to 3 carbon atoms, alkoxy having from 1 to 3 carbon atoms, phenoxy, or halo;

5 R¹¹ and R¹³ are each independently hydrogen, alkyl having 3 or 4 carbons, cycloalkyl having 5 or 6 carbon atoms, or R¹¹ and R¹³ are both phenyl;

R⁷ is O or NH;

R⁸ is hydrogen or methyl;

10 R⁹ is



R¹⁰ is hydrogen or methyl;

p is 0 or 1;

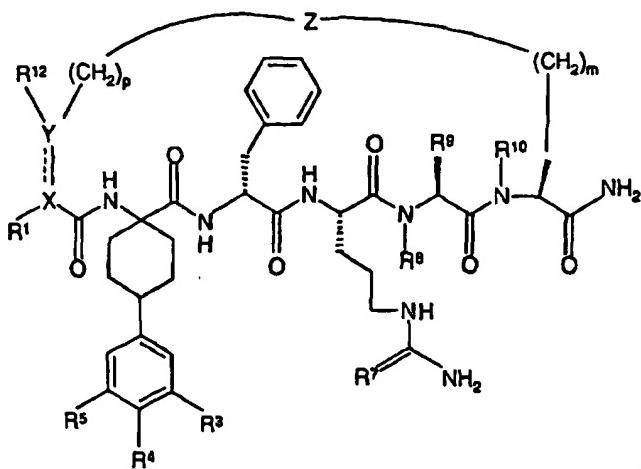
m is 0, 1, 2, or 3; and

15 Z is $\text{---C}=\text{O} \text{---NH---}$ or ---S---S--- ; and

R¹⁷ is hydrogen or lower alkyl

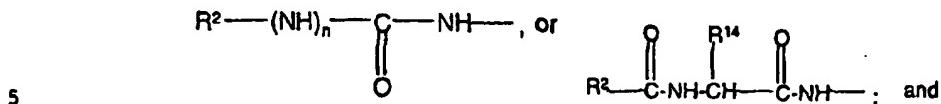
and pharmaceutically acceptable salts thereof.

20 2. A compound of the formula:



wherein

R^1 and R^{12} together with X and Y form a phenyl ring and X is C and Y is C, or
 R^1 is hydrogen,



R^{12} is hydrogen, with either X and Y being each C and the bond between X and Y being a double bond, or with X and Y being each CH and the bond between X and Y being a single bond;

10 R^2 is alkyl having from 1 to 5 carbon atoms, alkenyl having from 2 to 5 carbon atoms, or alkynyl having from 2 to 5 carbon atoms;

R^{14} is alkyl having from 1 to 5 carbon atoms;

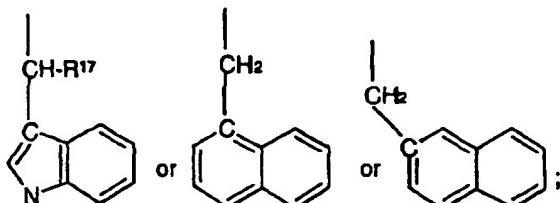
n is 0 or 1;

15 R^3 , R^4 and R^5 are independently hydrogen, halo, alkyl having from 1 to 4 carbon atoms, hydroxy, or alkoxy having from 1 to 4 carbon atoms; wherein when R^4 is not hydrogen, R^3 and R^5 are both hydrogen;

R^7 is O or NH;

R^8 is hydrogen or methyl;

R^9 is

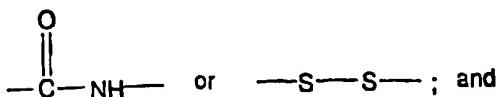


R^{10} is hydrogen or methyl;

p is 0 or 1;

m is 0, 1, 2, or 3; and

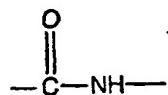
Z is



5

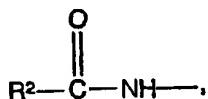
R¹⁷ is hydrogen or lower alkyl
and pharmaceutically acceptable salts thereof.

3. The compound of claim 2, wherein X and Y are each CH and the bond between X and Y is
10 a single bond; Z is



R^7 is O;

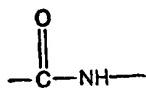
R^1 is



15 R² is alkyl having from 1 to 5 carbon atoms; and
R¹⁰ and R¹² are both hydrogen.

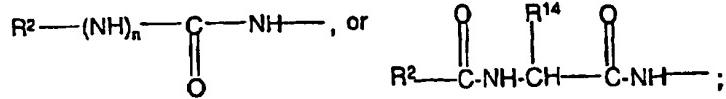
4. The compound of claim 3, Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Cit-Trp-Lys-NH₂.

- 20 5. The compound of claim 2, wherein Z is



R⁷ is NH;

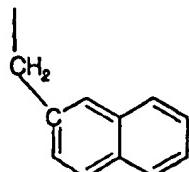
R' is hydrogen,



25 R² is alkyl; and

R^{10} and R^{12} are both hydrogen; and n and R^{14} are as defined in claim 2.

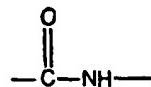
6. The compound of claim 5, wherein X and Y are each CH and the bond between X and Y is a single bond; n is 0; and R^9 is



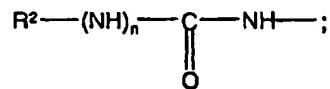
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7. The compound of claim 6, Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Arg-(2)Nal-Lys-NH₂ or penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Arg-N-methyl(2)Nal-Lys-NH₂.

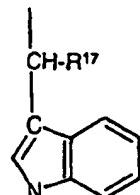
- 10 8. The compound of claim 5, wherein Z is



R^7 is NH; R^1 is



R^2 is alkyl; and R^{10} and R^{12} are both hydrogen; R^9 is



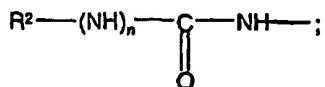
15

and R^{17} is as defined in claim 2.

- 20 9. The compound of claim 5, wherein X and Y are each CH and the bond between X and Y is a single bond; and one of R^3 , R^4 and R^5 is hydrogen, halo or alkyl and the remainder are hydrogen.

10. The compound of claim 9, Penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Arg-Trp-Lys-NH₂,
 penta-cyclo(Asp-Lys)-Asp-4-MeApc-(D)Phe-Arg-Trp-Lys-NH₂,
 penta-cyclo(Glu-Lys)-Glu-Apc-(D)Phe-Arg-Trp-Lys-NH₂,
 penta-cyclo(Asp-Orn)-Asp-Apc-(D)Phe-Arg-Trp-Orn-NH₂,
 5 penta-cyclo(Asp-Dbr)-Asp-Apc-(D)Phe-Arg-Trp-Dbr-NH₂,
 penta-cyclo(Asp-Dpr)-Asp-Apc-(D)Phe-Arg-Trp-Dpr-NH₂, or
 Ac-cyclo(Asp-Dpr)-Asp-Apc-(D)Phe-Arg-Trp-Dpr-NH₂.

11. The compound of claim 8, wherein X and Y are each CH and the bond between X and Y is a
 10 single bond; and R¹ is

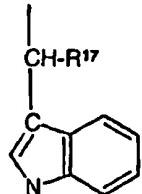


one of R³, R⁴ and R⁵ is alkoxy, and the remainder are hydrogen; and n is 0.

12. The compound of claim 11,

- 15 Penta-cyclo(Asp-Lys)-Asp-4-MeOAp-(D)Phe-Arg-Trp-Lys-NH₂,
 penta-cyclo(Asp-Lys)-Asp-4-EtOAp-(D)Phe-Arg-Trp-Lys-NH₂,
 penta-cyclo(Asp-Lys)-Asp-4-iPrOAp-(D)Phe-Arg-Trp-Lys-NH₂,
 penta-cyclo(Asp-Lys)-Asp-3-MeOAp-(D)Phe-Arg-Trp-Lys-NH₂,
 penta-cyclo(Asp-Lys)-Asp-4-OHAp-(D)Phe-Arg-Trp-Lys-NH₂, or
 20 penta-cyclo(Asp-Lys)-Asp-4-ClAp-(D)Phe-Arg-Trp-Lys-NH₂.

13. The compound of claim 8, wherein each of R¹, R³, R⁴, R⁵, R⁸ and R¹⁰ is hydrogen;
 R⁷ is NH;
 R⁹ is



25

P is 0; and R¹⁷ is as defined in claim 2.

14. The compound of claim 14, Cyclo(succinic acid-Lys)-succinic acid-Apc-(D)Phe-Arg-Trp-Lys-NH₂,

Cyclo(maleic acid-Lys)-maleic acid-Apc-(D)Phe-Arg-Trp-Lys-NH₂,

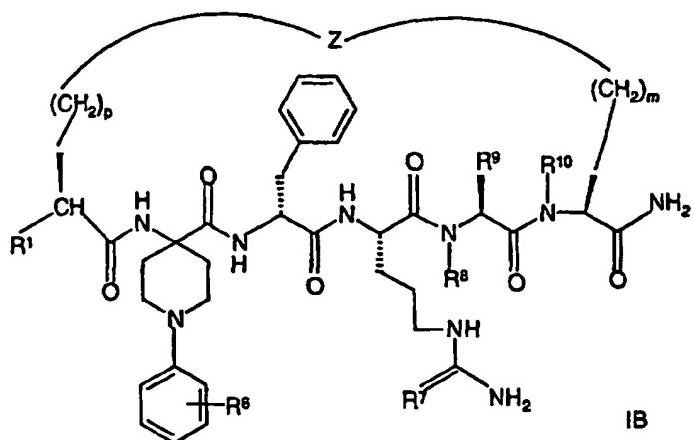
Cyclo(succinic acid-Dpr)-succinic acid-Apc-(D)Phe-Arg-Trp-Dpr-NH₂, or

5 Cyclo(maleic acid-Dpr)-maleic acid-Apc-(D)Phe-Arg-Trp-Dpr-NH₂.

15. The compound of claim 2, wherein R¹ and R¹² together with X and Y form a phenyl ring.

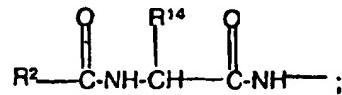
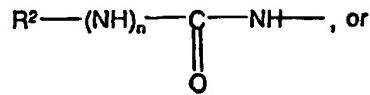
16. The compound of claim 15, Cyclo(phthalic acid-Lys)-phthalic acid-Apc-(D)Phe-Arg-Trp-Lys-NH₂, or cyclo(phthalic acid-Dpr)-phthalic acid-Apc-(D)Phe-Arg-Trp-Dpr-NH₂, or
10 Ac-Nle-cyclo(Cys-Cys)-Cys-Apc-(D)Phe-Arg-Trp-Cys-NH₂.

17. A compound of the formula:



15 wherein

R¹ is hydrogen,



20 R² is alkyl having from 1 to 5 carbon atoms, alkenyl having from 2 to 5 carbon atoms, or alkynyl having from 2 to 5 carbon atoms;

R¹⁴ is alkyl having from 1 to 5 carbon atoms;

n is 0 or 1;

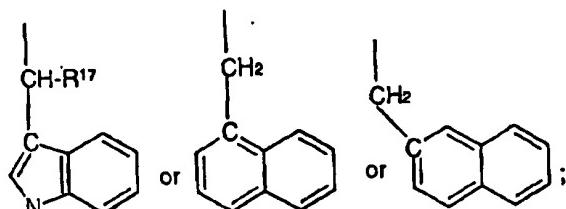
- 221 -

R^6 is hydrogen, alkyl having from 1 to 3 carbons, alkoxy having from 1 to 3 carbons, phenoxy, or halo;

R^7 is O or NH;

R^8 is hydrogen or methyl;

5 R^9 is

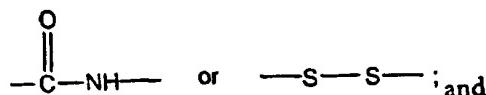


R^{10} is hydrogen or methyl;

p is 0 or 1;

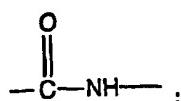
10 m is 0, 1, 2, or 3; and

Z is



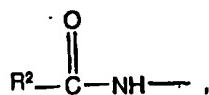
R^{17} is hydrogen or lower alkyl and pharmaceutically acceptable salts thereof.

15 18. The compound of claim 19, wherein Z is



R^7 is NH;

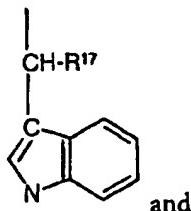
R^1 is



20 R^2 is alkyl;

R^8 and R^{10} are each hydrogen; and

R^9 is



R¹⁷ is as defined in claim 2.

19. The compound of claim 18, wherein R⁶ is hydrogen or alkyl.

5

20. The compound of claim 19, Penta-cyclo(Asp-Lys)-Asp-Appc-(D)Phe-Arg-Trp-Lys-NH₂,

Penta-cyclo(Asp-Lys)-Asp-2-MeAppc-(D)Phe-Arg-Trp-Lys-NH₂,

Penta-cyclo(Asp-Lys)-Asp-2-iPrAppc-(D)Phe-Arg-Trp-Lys-NH₂,

Penta-cyclo(Asp-Lys)-Asp-3-MeAppc-(D)Phe-Arg-Trp-Lys-NH₂, or

10 Penta-cyclo(Asp-Lys)-Asp-4-MeAppc-(D)Phe-Arg-Trp-Lys-NH₂.

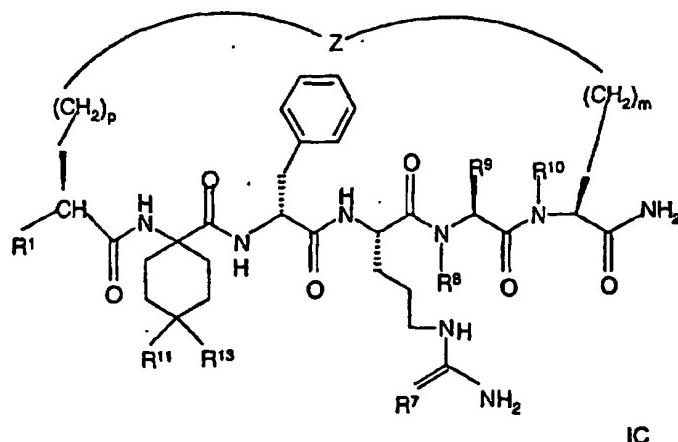
21. The compound of claim 19, wherein R⁶ is halo.

- 15 22. The compound of claim 23, Penta-cyclo(Asp-Lys)-Asp-4-ClAppc-(D)Phe-Arg-Trp-Lys-NH₂.

23. The compound of claim 20, wherein R⁶ is alkoxy or phenoxy.

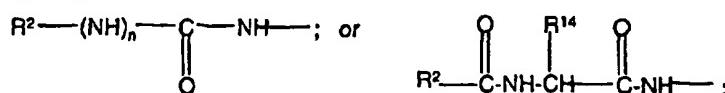
- 20 24. The compound of claim 24, Penta-cyclo(Asp-Lys)-Asp-4-PhOAppc-(D)Phe-Arg-Trp-Lys-NH₂, or Penta-cyclo(Asp-Lys)-Asp-3-MeO-Appc-(D)Phe-Arg-Trp-Lys-NH₂.

25. A compound of the formula:



wherein

R^1 is hydrogen,



5 R^2 is alkyl having from 1 to 5 carbon atoms, alkenyl having from 2 to 5 carbon atoms, or
alkynyl having from 2 to 5 carbon atoms;

R^{14} is alkyl having from 1 to 5 carbon atoms;

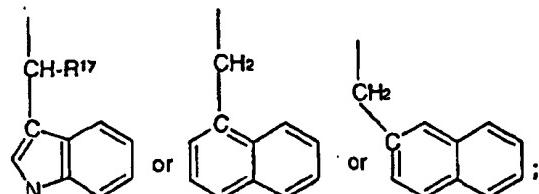
n is 0 or 1;

10 R^{11} and R^{13} are each independently hydrogen, alkyl having 3 or 4 carbon atoms, or
cycloalkyl having 5 or 6 carbon atoms or R^{11} and R^{13} are both phenyl;

R^7 is O or NH;

R^8 is hydrogen or methyl;

R^9 is

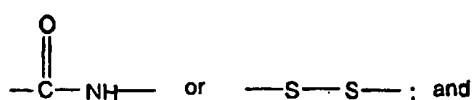


15 R^{10} is hydrogen or methyl;

p is 0 or 1;

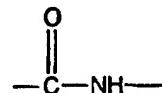
m is 0, 1, 2, or 3; and

Z is



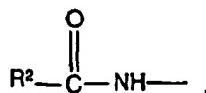
R^{17} is hydrogen or lower alkyl and pharmaceutically acceptable salts thereof.

26. The compound of claim 25, wherein Z is.



5 R^7 is NH;

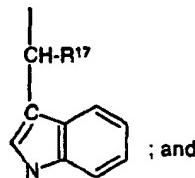
R^1 is



R^2 is alkyl;

R^8 and R^{10} are each hydrogen; and

10 R^9 is



R^{17} is hydrogen or lower alkyl.

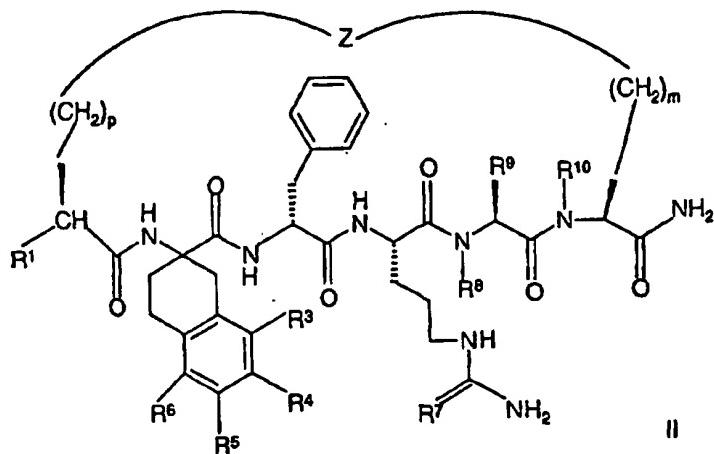
27. The compound of claim 26, wherein one of R^{11} and R^{13} is alkyl or cycloalkyl and the other
15 is hydrogen.

28. The compound of claim 27, penta-cyclo(Asp-Lys)-Asp-Achc-(D)Phe-Arg-Trp-Lys-NH₂,
or penta-cyclo(Asp-Lys)-Asp-Abc-(D) Phe-Arg-Trp-Lys-NH₂.

20 29. The compound of claim 26, wherein R^{11} and R^{13} is phenyl and the other is hydrogen or
phenyl.

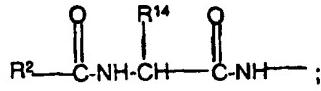
30. The compound of claim 29, penta-cyclo(Asp-Lys)-Asp-4-Adpc-(D)Phe-Arg-Trp-Lys-NH₂.

31. A compound of the formula:



wherein

R^1 is hydrogen,



5

R^2 is alkyl having from 1 to 5 carbon atoms, alkenyl having from 2 to 5 carbon atoms, or alkynyl having from 2 to 5 carbon atoms;

R^{14} is alkyl having from 1 to 5 carbon atoms;

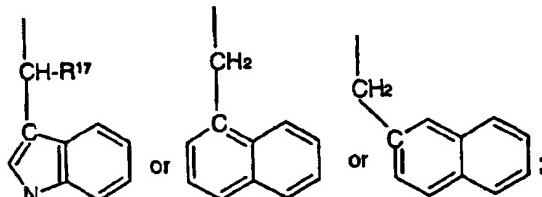
n is 0 or 1;

10 one of R^3 , R^4 , R^5 and R^6 is hydrogen, halo, alkyl having from 1 to 3 carbon atoms, or alkoxy having from 1 to 3 carbon atoms, and the remainder are hydrogen;

R^7 is O or NH;

R^8 is hydrogen or methyl;

R^9 is



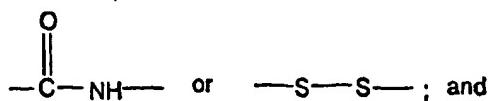
15

R^{10} is hydrogen or methyl;

p is 0 or 1;

m is 0, 1, 2, or 3; and

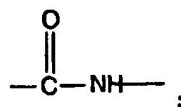
Z is



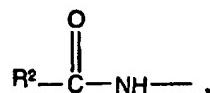
R^{17} is hydrogen or lower alkyl and pharmaceutically acceptable salts thereof.

32. The compound of claim 31, wherein Z is

5



R^1 is

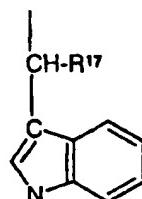


R^2 is alkyl;

10 $\text{R}^3, \text{R}^4, \text{R}^5, \text{R}^8$ and R^{10} are each hydrogen;

R^6 is hydrogen, halo, alkyl having from 1 to 3 carbon atoms, or alkoxy having from 1 to 3 carbon atoms; and

R^9 is



15 and R^{17} is as defined in claim 31.

33. The compound of claim 32, wherein R^7 is NH.

20

34. The compound of claim 32, wherein R^6 is hydrogen or alkyl.

35. The compound of claim 34, penta-cyclo(Asp-Lys)-Asp-(D,L)-Atc-(D)Phe-Arg-Trp-Lys-NH₂,
penta-cyclo(Asp-Lys)-Asp-5-Me-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂,

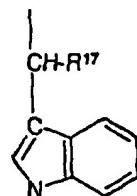
penta-cyclo(Asp-Lys)-Asp-5-Et-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂, or
 penta-cyclo(Asp-Lys)-Asp-5-iPr-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂.

36. The compound of claims 31 to 33, wherein R⁶ is halo.
- 5
37. The compound of claim 36, penta-cyclo(Asp-Lys)-Asp-5-BrAtc-(D)Phe-Arg-Trp-Lys-NH₂, or penta-cyclo(Asp-Lys)-Asp-5-ClAtc-(D)Phe-Arg-Trp-Lys-NH₂.
- 10
38. The compound of claims 31 to 33, wherein R⁶ is alkoxy.
39. The compound of claim 38, penta-cyclo(Asp-Lys)-Asp-5-MeO-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂,
 penta-cyclo(Asp-Lys)-Asp-5-EtO-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂, or
 penta-cyclo(Asp-Lys)-Asp-5-iPrO-(D,L)Atc-(D)Phe-Arg-Trp-Lys-NH₂.
- 15
40. The compound of claims 31 or 32, wherein R⁷ is O and R⁶ is halo.
41. The compound of claim 40, penta-cyclo(Asp-Lys)-Asp-5-BrAtc-(D)Phe-Cit-Trp-Lys-NH₂, or penta-cyclo(Asp-Lys)-Asp-5-ClAtc-(D)Phe-Cit-Trp-Lys-NH₂.
- 20
42. The compound of claim 31, wherein Z is -S-S- ;
 R¹ is

$$\begin{array}{c} \text{R}^2-\text{(NH)}_n-\text{C}(=\text{O})-\text{NH}- \\ \text{or} \end{array}$$

$$\begin{array}{c} \text{R}^2-\text{C}(=\text{O})-\text{NH}-\text{CH}-\text{C}(=\text{O})-\text{NH}- \end{array}$$
 R³, R⁴, R⁵, R⁸ and R¹⁰ are hydrogen;
 R⁶ is hydrogen or halo;
 R⁷ is NH;
 R⁹ is

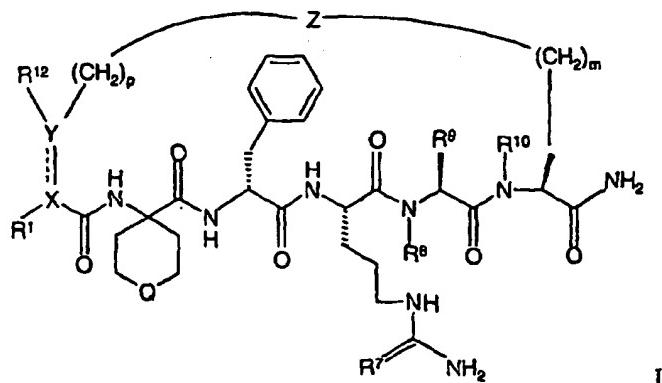
- 228 -



wherein R¹⁷ is as above.

43. The compound of claim 42, Ac-Nle-cyclo(Cys-Cys)-Cys-(D,L)Atc-(D)Phe-Arg-Trp-Cys-NH₂,
 5 penta-cyclo(Cys-Cys)-Cys-5-Br(D,L)Atc-(D)Phe-Arg-Trp-Cys-NH₂,
 penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Ala-Trp-Lys-NH₂, or
 penta-cyclo(Asp-Lys)-Asp-Apc-(D)Phe-Arg-(2S,3S) beta methyl-Trp-Lys-NH₂.

- 10 44. A process for the preparation of a compound of the formula:



wherein

R¹ to R¹², m, p, Q, X, Y and Z are as defined in claims 1 to 43

by formation of a lactam bond or a disulfide bond at the Z position of the precursor linear

15 peptides.

45. A pharmaceutical composition comprising compounds according to any one of claims 1-43 and a therapeutical inert carrier.

46. Use of compounds according to any of claims 1 to 43 for the preparation of medicaments for the treatment and/or prophylaxis of diseases which are associated with melanocortin-4-receptor activity.

5 47. A method for the treatment and/or prophylaxis of diseases which are associated with melanocortin-4-receptor activity, which method comprises administering a compound according to any of claims 1 to 43 to a human being or animal.

48. The use or method of claims 46 or 47 wherein the disease is obesity.

10

49. A compound to any one of claims 1-43 when manufactured by a process according to claim 44.

15 50. Compounds according to any of claims 1 to 43 for use as therapeutic active substances, particularly as therapeutic active substances for the treatment and/or prophylaxis of diseases which are associated with melanocortin-4-receptor.

51. The novel compounds, novel pharmaceutical compositions and processes as well as the use of such compounds substantially described herein before.

20

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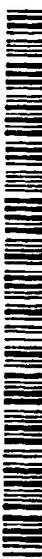
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(54) Title: CYCLIC PEPTIDES HAVING MELANOCORTIN-4 RECEPTOR AGONIST ACTIVITY

(57) Abstract: The present invention refers to peptides cyclized via disulfide or lactam bridges having melanocortin-4 receptor (MC4-R) agonist activity useful for treatment of obesity.

INTERNATIONAL SEARCH REPORT

Inter	nternational Application No
PCT/EP 01/09630	

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C07K14/68 A61K38/34 A61P3/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, MEDLINE, BIOSIS, WPI Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FAN W ET AL: "ROLE OF MELANOCORTINERGIC NEURONS IN FEEDING AND THE AGOUTI OBESITY SYNDROME" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 385, 9 January 1997 (1997-01-09), pages 165-168, XP002072475 ISSN: 0028-0836 cited in the application the whole document ---	1-50 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *'A' document defining the general state of the art which is not considered to be of particular relevance
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- *'O' document referring to an oral disclosure, use, exhibition or other means
- *'P' document published prior to the international filing date but later than the priority date claimed

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BEDNAREK M A ET AL: "ANALOGS OF MTII, LACTAM DERIVATIVES OF ALPHA-MELANOTROPIN, MODIFIED AT THE N-TERMINUS, AND THEIR SELECTIVITY AT HUMAN MELANOCORTIN RECEPTORS 3, 4, AND 5" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, vol. 261, no. 1, 1999, pages 209-213, XP002159223 ISSN: 0006-291X the whole document ----	1-50
A	ADAN E.A.: "Characterization of melanocortin receptor ligands on cloned brain melanocortin receptors and on grooming behaviour of rats" EUROPEAN JOURNAL OF PHARMACOLOGY, vol. 378, 1999, pages 249-258, XP002192102 the whole document ----	1-50
A	BENOIT E.A.: "A novel selective melanocortin-4 receptor agonist reduces food intake in rats and mice without producing aversive consequences" J.NEUROSCIENCE, vol. 20, no. 9, 1 May 2000 (2000-05-01), pages 3442-3448, XP002192127 the whole document -----	1-50

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Continuation of Box I.2

Claims Nos.: 51

Present claim 51 lacks any specific technical feature allowing for a search beyond the scope of the preceding claims. Furthermore, due to the use of the expression "substantially" said claim also lacks clarity (Article 6 PCT). Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely the claims 1-50.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.